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PREFACE

A long time has passed from the days when the modern ophthalmology was introduced into Japan, meanwhile there must have been not a few results of research published by our ophthalmologists, which should be a great contribution to ophthalmology in the world. To my great regret, however, there has been no organ, which imparts the full idea of the descriptions, to the overseas ophthalmologists, notwithstanding the presence of short abstracts without illustrations, owing to the fiinancial difficulties in our ophthalmological society.

So I laid a plan to publish the Japanese Journal of Ophthalmology before my respected friend Mr. Mampei Suzuki, President of the Sankyo Pharmaceutical Company, who encouraged and promised me financial support for it with immense understanding and goodwill.

Nowadays we have in Japan four monthlies, Acta Ophthalmologica Japonica, Ganka-Rinshoiho, Japanese Journal of Clinical Ophthalmology and Folia Ophthalmologica Japonica, original essays given in them numbering eight hundred every year. It is our ultimate object to insert all of them into this Japanese Journal of Ophthalmology, but we have now no space enough to do it. So we expect to select from sixty to eighty essays yearly out of the four journals to introduce them, laying emphasis on the illustrations.

Would that this Journal serve as an informant of the present state of researches in ophthalmology in our country.

Editor H. Hagiwara.

Felicitations on Publication of the Japanese Journal of Ophthalmology

Difficulties in reading and writing Japanese have been the cause of difficulties in understanding scientific literatures written in Japanese for the learned people of foreign countries.

With the intention of substituting these complicated Japanese, letters for new ones, easier to read and write, I have been laboring for these thirty years and recently obtained some plan, which, however, is not yet in use.

On the contrary, the bulk of European and American literatures are written with Latin or similar letters, and it is not so difficult to read and understand them.

For this reason we have been in a favourable condition of introducing foreign sciences into Japan, but the progress of the Japanese has had no ample chance to be introduced to foreign countries.

Dr. H. Hagiwara, Prof. of the University of Tokyo, formed a plan to issue a journal of the ophthalmological researches in Japan written in English in order to correct this unequal interchange of scientific culture, and it has now materialized.

I hope that this significant and precious work will continue forever and become prosperous with the years.

Emeritus Prof. of the University of Tokyo
Shinobu Ishihara

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AN ANALYSIS OF THE SPATIAL SUMMATION OF THE SENSITIVITY OF THE RETINA

Shin-ichi SHIKANO

Department of Ophthalmology, School of Medicine, University of Tokyo

(1)

The threshold brightness of a test patch required to be just visible within limits depends on its size—a large patch requiring a smaller brightness than a small patch.

The Liminal Brightness×Area=Constant (Ricco's Law) holds moderately well, i.e. the threshold stimulus is determined by the amount of light entering the eye.

This retinal summation is explained by the fact that the rods are capable of summating their effects, connecting to the ganglion cells by way of diffuse bipolars whose dendritic endings make synaptical contacts with many rod spherules.

Then, whether this law about the spatial summation of the sensitivity can be applied or not, to photopic vision and to the sensitivity of the cones in the fovea, wherin, according to Polyak, the bipolars and the ganglion cells connect each other one to one, is a question that must be answered by further experiments. The first experiment was designed to solve this question.

Observer, Apparatus and Method

The greater part of the work was done with the writer as observer. The apparatus was composed from the Nagel's adaptometer (A), a box (B), a fixation point (C), a secter (D) and so on (Fig. 1). The test patches were round holes of the metalic plates placed on the surface of Nagel's adaptometer. The number of those holes were 32 and their visual angles observed from 20 cm. distant were as follows.

B was a black bottomles box (10×10×10 cm.). The sector, whose two white wings had a 120° angle and was illuminated (150 rlux) diagonally, moved with slow velocity (2.2 cycles per minute), i.e. the observer adapted to 150 rlux for 9.1 sec. and watched the test patch in the dark at regular intervals intermittently for 4.5 sec. The thres-

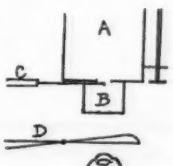

Fig. 1 A—Nagel's Adaptometer
B—A bottomless black box
C—A fixation point
D—A secter
The distance between the eye and the test patch was 20 cm.

0.5mm	4′25″	11 mm	1°34.5′	30 mm	4°16′
1	8′06″	12	1°43.5′	34	4°58′
2	17.5′	13	1°51.5′	40	5°59.6′
3	26′	14	2°00′	45	6°02′
4	34.5′	15	2°09′	59	7°01.5′
5	43′	16	2°17.5′	60	8°21′
6	52′	17	2°26′	70	9°39′
7	1°00′	18	2°34.5′	80	10°54.5′
8	1°09′	19	2°43′	90	12°07′
9	1°17.5′	20	2°51.5′	100	13°17′
10	1°26′	21	3°34′		

hold brightness was measured by means of the Nagel's adaptometer, of which the maximum brightness was made 7.08 rlux.

The experiment with the red light (above 660 mμ) in scotopic vision was also tried.

Results (Fig. 2)

In photopic vision, the corelation curve between the logarythm of test patches (in the limitation of visual angles 4′25″5°) and the logarythm of the threshold brightness showed a straight line. Accordingly the Ricco's law holds well in photopic sensitivity as well as in scotopic sensitivity. The ascending of the curve out of 5° was due to the irradiation in the box B.

The curve of the experiment with the red light in scotopic condition showed also a straight line, but it could be divided into two parts. The writer attributed its slower part to the participation of rods in the threshold of the red sensitivity.

Fig. 3 showed the usual relationship between the extention of test patches and the threshold brightness in scotopic condition.

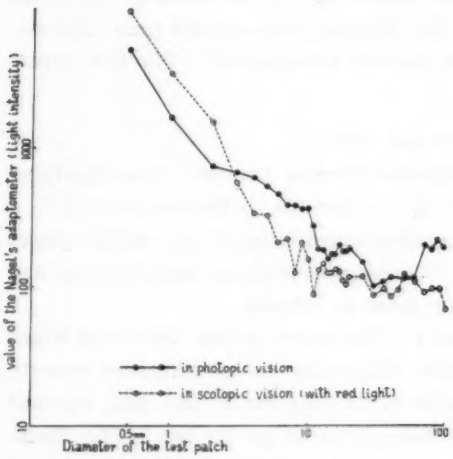

Fig. 2 The spatial summation of the sensitivity of cones. The line of solid circles represents the threshold brightness in photopic vision and the line of the clear circles, the threshold brightness for red light (above660 mμ) in scotopic vision. The ordinate represents the logarythm of the scale of the Nagel's adaptometer and the abscissa, the logarythm of the diameter of the test patch.

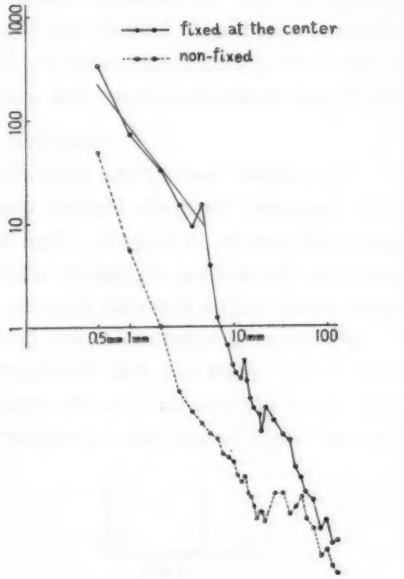

Fig. 3 The spatial summation of the sensitivity of rods measured as usual in scotopic vision. (The line of solid circles) The ordinate represents the logarythm of the scale of Nagel's adaptometer and the abscissa, the logarythm of the diameter of the test patch.
The line of clear circles represents thresholds without fixation.

(2)

The writer studied further the physiological mechanism of the spatial summation. Ricco's law treats of the two-dimensional extension of test patches. If the test patch stretches one-dimensionally, what alteration would take place about this law?

Method

In scotopic vision (after 30 minutes dark adaptation), the threshold sensitivities were measured as follows.

a) The test patch was 1 mm. in width and its length varied from 1 mm. to 50 mm. (1,2,4,8,10,12,14,16,18,20,25,30,35,40,45,50 mm.), and the fixation point was placed at the centre.

b) The test patch was 5 mm. wide and its length varied as above mentioned.

Results

The relationship between the logarythm of the threshold brightness and the logarythm of length of the test patch was illustrated by Fig. 4. It showed that a long patch required a smaller brightness proportionally than a short patch within the limit of 16 mm length. But, beyond 20 mm in length, the threshold brightness decreased no more.

The reason of this phenomena was as follows; when man watched the fixation point

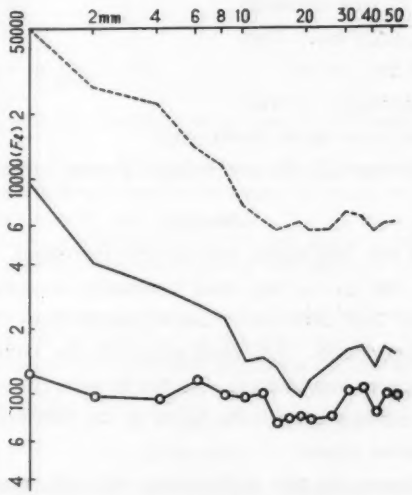

Fig. 4 The relationship between the threshold brightness and the length of the test patch. The black line represents the threshold brightness of the centre for the test patch which was 1 mm in width, the line of clear circles, those for the test patch which was 5 mm wide and the dotted line, those for the red test patch which was 1 mm wide.
The ordinate represents the logarythm of the scale of Nagel's adaptometer and the abscissa, the the logarythm of the length of the test patch.

at the center of the test patch, the peripheral end was observed at small brightness, but the central part could not be seen, being melted together with the surrounding darkness. The more the brightness of the test patch increased, the more distinct the peripheral end became and the brightness disappeared decreasingly towards the centre.

The threshold brightness at the central part, requiring to be just visible, became in this way large. The plotted points in Fig. 4 showed this threshold brightness at the center. It can be seen clearly that the length of the test patch, when large enough, does not influence the central threshold sensitivity.

(3)

What does this result on the Rico's law mean, which treats of the corelation between the two-dimensional extention of the stimulus and the threshold sensitivity? The writer tried further an experiment about this law, placeing a seperating dark zone in the test patch between the center and the periphery.

Method

A black paper such as Fig. 5 was stuck on a plate glass, which was put on the stimulating surface of the Nagel's adaptometer.

a) A in Fig. 5 was 1cm, B was 1.5 cm, D was 0.25 cm. and C was variable (7 kind as 2, 2.5, 3, 3.5, 4, 4.5, 5 cm.) The fixation point was set at the centre.

b) B was 2 cm, accordingly D was 0.5 cm. and C was variable as above mentioned.

c) A was 0.5 cm, B was 1.5 cm, accordingly D was 0.5 cm, and C was variable.

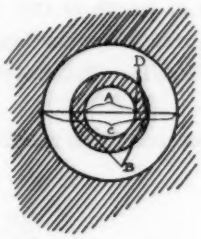

Fig. 5 The test patch used in experiment 3.

Results

As the brightness of the test patch was slowly increased, the large test patch was first observed as a disk, not as a ring, and gradually an obscure dark part became noticeable in the center of this disk. At a higher brightness, the border of the central circle became finally observable. On each point of the curve in Fig. 6 the threshold brightness of the inner circle was plotted. As can be seen from this curve, the threshold brightness of the inner circle required its figure to be just visible, not influenced from the enlargment of the outer circle.

The threshold brightness for the outer circle was also measured and plotted -x- in Fig. 6. It can be seen cleary that this plotted line shows the same descendence to the curve in Fig. 3 of the experiment (1) which dealt with the single round test patch.

Namely, the decrease of the threshold brightness in proportion to the enlargment of the test patch, as it has been showed by the Ricco's law, was found to be caused by the increase of the sensitivity at the border of the test patch and not due to a

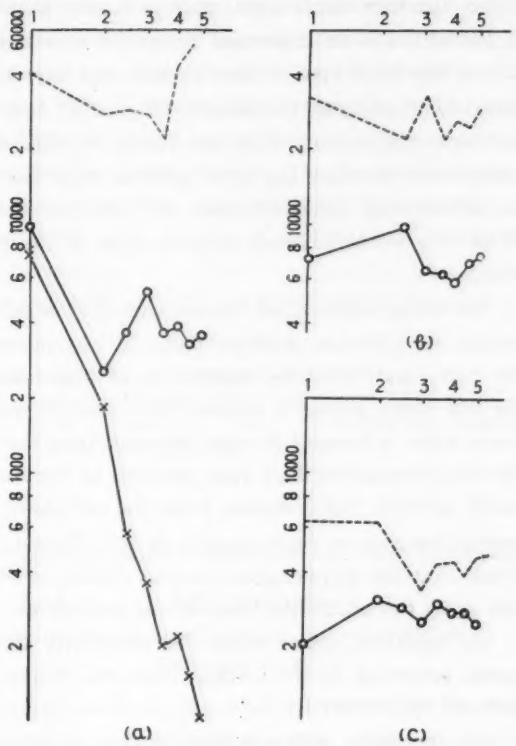

Fig. 6 The relationship between the threshold brightness of the centre and the periphery of the test patch.

a) The line of clear circles represents the threshold brightness at the centre of the test patch as fig. 5 in which the non-stimulative zone (D) was 0.25 cm. and the line of -x-, the threshold brightness of the outer circle.

b) The threshold brightness at the centre for the test patch in which D was 0.5 cm.

c) The threshold brightness at the center for the test patch in which the central circle (A) was 0.5 cm. and D is 0.5 cm.

The ordinate represents the logarythm of the scale of Nagel's adaptometer and the abscissa, the logarythm of the diameter of the outer circle

Each dotted line represents the threshold brightness for the red test patch.

true summation of the sensation.

Comment

That a central scotom appears in the scotopic vision is a well known fact in the ophthalmological physiology. It is an important consideration to the analysis of the mechanism of the spatial summation of the sensitivity that this physiological scotom is a negative one. The sensation in the negative scotom is quite abscent (neither black nor gray) such as in the Mariotte blind spot.

There have been many experiments and theories about the state of subjective

imperceptibility of the Mariotte blind spot, and it is sure that the sensation of the surrounding area of the retina is an important factor for this imperceptibility. Volkmann demonstrated that the blind spot is filled in with the surrounding sensation.

It would be natural to think, that the same hold good of this negative scotom at the fovea. In the previous explanations about the results of experiments on the spatial summation of the sensitivity, the fact has been ignored, that this physiological scotom is absorbed by the surrounding sensation and the threshold sensitivity at the fovea has been considered as if it would increase in proportion to the peripheral sensitivity of the stimulated retina.

In experiment 2, the writer carried out the measurement of the threshold sensitivity at the fovea, restricting the influence of this "filling in" by means of one-dimensional extending of the test patch and found the sensitivity of the fovea not influenced by the stretching of the test patch beyond a certain limitation (10 mm.), i.e. the darkness of the upper and lower sides influenced it more strongly than the end of the test patch. In experiment 3, the non-stimulative dark ring inserted at the middle zone of the test patch restricted almost perfectly the influence from the periphery.

The so-called spatial summation dealt roughly in the Ricco's law (or the Piper's law) is due to the fact that the physiological central scotom in scotopic vision is filled in with the sensation from the peripheral zone of the stimulated retina. These laws show therefore only the condition, under which the sensitivity of the para-central part of the retina increases, according to the distance from the center, and no true rule of the spatial summation of the sensitivity.

The number of rods increases, within a limit, with a rapid ascent from the center to the periphery (Φsterberg). When test patch extends two-dimensionaly, the number of the rods included in the stimulated retina becomes a colossal sum. Recently C. Haig made a new law, $I \times A/S = C$ (it concerned the separated part of the retina), calculated from the Φsterbergs chart about the rod-distribution and the Polyaks data about the diameter of rods and cones (A=area, I=intensity of the stimulus, S=diameter of the rod, C—constant, but variable according to the situation in the retina).

But, the writer is of the opinion that his law could hold well within a far larger range at the central vision where Ricco's law is concernd and might mean the analysis of Ricco's law itself, when it is considered that the increase of the sensitivity is related only to the peripheral, not to the total area.

Summary

The writer experimented on the spatial summation of the sensitivity and examined the physiological mechanism of Ricco's law.

It has been said that Ricco's law is due to the function of the rod-system, which converges impulses from a wide area on the retina, into a single optic nerve by way of the synaptical connection between rods, bipolars and ganglion cells.

The writer found that the law of the spatial summation of the sensitivity holds also well in the photopic vision and by the red stimulus, therefore the spatial summation is not due only to the connection form of the rod-system in the retina.

Further it was found that in the scotopic vision, the spatial summation was, so to say, an illusion owed to the physiological scotom, which is negativ and absorbed by the surrounding sensation. On this account, the increase of the sensitivity in the central retina in propotion to the enlargement of the test patch, is the same as the increase of the sensitivity at the peripheral part of the stimulated retina according to its distance from the center.

(Published in Acta. O. Jap. 58:167, 1954, and 58:479,.1954 in Japanese)

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PHYSICO-CHEMICAL STUDIES OF THE VITREOUS

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The problem of the structure of the adult vitreous body has long been debated and it has been proved that it has a fibrous structure. However, the question which cell layers contribute to its structure, is still now unsolved. The present work was started with the intention of identifying the fibrous components of the vitreous which had been revealed by the electron microscopic examination.

Preparation of specimens. Materials used were prepared in the following manner. The vitreous bodies were removed from the eyes of the horse, bovine, and rabbit within 0-24 hours after death of the animals by making an equatorial cut through the tissues and allowing the vitreous to fall away from the eye.

The vitreous was then fragmented in a blender for 2 minutes in the cold. The clear, aqueous supernatant obtained by centrifuging at 1460 G. for 15-40 minutes was then centrifuged at 20,000 G. for 60 minutes and a small amount of sediment was obtained. These high speed sediments were collected and used for electron microscopy and X-ray analysis. The insoluble membraneous residue prepared by filtration of the fresh vitreous was used for infrared absorption.

Electron microscopic observation. The high speed sediment described above was resuspended in distilled water and prepared for examination by depositing a drop on supporting films of the specimen grid and blotting them off with filter paper after about 30 seconds. All preparations were shadowed with chromium. The vitreous treated

by ultrasonics (560 KC. 1000, 570 Watt, for 2 minutes.) were observed in the same manner.

Electron micrograph of the vitreous as shown in Fig. 1 revealed chiefly two distinct types of fibrils which were common to horse, bovine, and rabbit. (Fig. 1)

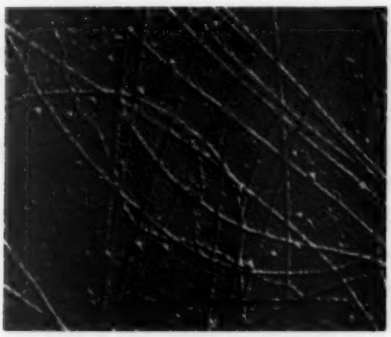

Fig. 1 Fibrils of bovine vitreous Cr. shadowed.

The first type of fibril, termed A fibril, was represented by a long, unbranched, non striated fibril, curving in a relatively smooth arc. The fibril width ranged from 210 to 330 Å, averaging 260 Å. Some of them ran in touch with each other, appearing to be a new large fiber.

The second type of fibril, termed B fibril, was bent more crookedly and was clearly striated, appearing to be a chain formed of fine granules. The fibril width ranged from 230 to 290 Å, averaging 250 Å. No branching or fraying was observed. The axial periodicity varied between 490 and 980 Å, averaging 620 Å. Interperiod structure was not clear.

The ratio of the number of A : B was 26 : 1 in the bovine vitreous.

Fibrils of the vitreous treated by ultrasonics were strongly fragmented.

Several specimens were treated with trypsin, hyaluronidase and formaldehyde, and it was proved that these chemicals do not change the form of the two fibrous components.

Usual collgen fibers described by Matoltsy et al[1], were excluded almost completely in these specimens.

X-ray analysis. Instead of the material used by Pirie et al[2], the high-speed sediment described above, the Achilles tendon of a rabbit, and the substantia propria of the bovine cornea were dried and used for X-ray analysis. Several specimens were prepared from these materials, after 1.5 minutes' extraction with boiling ether. X-ray diagrams were obtained, using a photographic method with filtered copper radiation, at a film distance of 2-12 cm. X-ray reflections were also recorded on the chart of Philips Geiger Counter X-ray Spectrometer in the range of 3° to 50° (2θ). Filtered copper radiation, 35 KV, 15 MA, scan speeds 2 per minute, angular aperture 1, receiving slit 0.006 inch, time constant 4 second.

The X-ray pattern obtained consisted of a few, relatively sharp interferences, together

with broad amorphous and halos, and this was essentially the same for the vitreous the tendon, and the cornea.

Several specimens were stretched and photographed with "fiber axis" perpendicular to the X-ray beam. The characteristic of the patterns of the stretched vitreous is the equatorial arc at about 11.5 Å (at the normal humidity) and the meridional arc at 2.9 Å. This sharp, equatorial arc is given by a plane whose interplaner spacing varies from 11.0 Å up to about 12.5 Å, depending upon the amount of water held by by the protein. The spacings of 11.5 Å and about 4.5 Å were well checked with the X-ray spectrogram. The diffraction patterns are schematically reproduced in Figs. 2,3, and 4. The general agreement of the spacings of the X-ray reflections with those of the tendon and the substantia propria of the cornea provides support for the view that the protein of the vitreous belongs to the collagen type. (Fig. 2, Fig. 3, Fig. 4)

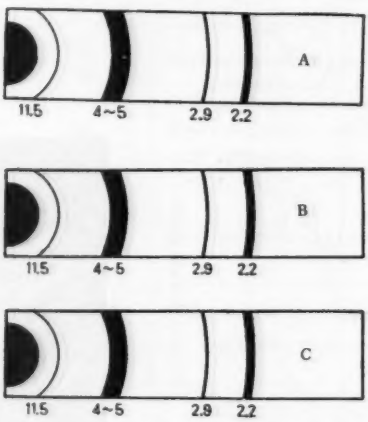

Fig. 2 X-ray diagrams of vitreous, tendon, and substantia propria of cornea. A: vitreous, B: tendon, C: substantia propria of cornea. Schematically reproduced from the data which were obtained at a specimen-film distance of 4 and 12 cm. Cu radiation, Ni filtered. (Å)

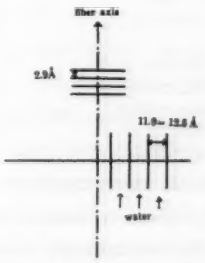

Fig. 3 Variation of interplaner spacing by hydration, Vitreous: 11.0~12.5 Å, tendon: 11.0~13.2, substantia propria of cornea: 11.0~13.1Å

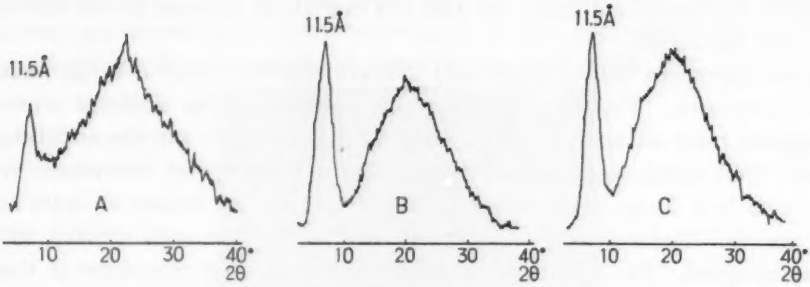

Fig. 4 X-ray diffraction spectrograms of vitreous, tendon. and substantia propria of cornea at the normal humidity. A: vitreous, B: tendon, C: substantia propria of cornea. Cu radiation, Ni filtered.

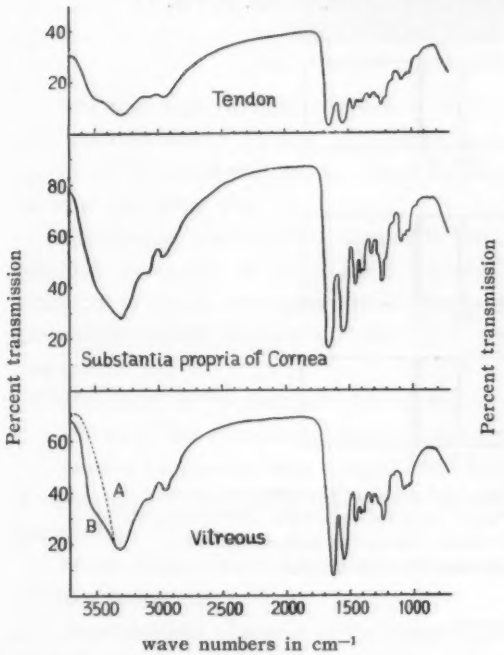

Fig. 5 Infrared spectra for vitreous, tendon, and substantia propria of cornea. Curve A, conc. H_2SO_4-dried vitreous; Cuve B, vitreous under water vapourof 9.5 mmHg. The thickness of the specimens is different with one another.

Fig. 6 Fibrils of the tendon of a rat embryo, weighing 3.5 g. Cr. shadowed.

Infrared absorption. The insoluble membraneous residue of the vitreous described above was immersed in a 5000 T.U./c.c. hyaluronidase solution buffered to pH 6.0 and incubated at 20°C for 24 hours. The membrane was then picked up, immersed in a saline solution in the cold for 7 weeks, and finally washed well in distilled water. The infrared spectrum was measured with this membrane in the range of 2-15 μ by a Perkin-

Elmer Spetrophotometer (Model 21). Specimens were also prepared from the Achilles tendon of a rabbit and the substantia propria of the bovine cornea in the same manner.

In all the spectra, there are several strong absorption bands which may be correlated with those of known chemical groupings, such as given in Table 1. In addition, bands at 1210, 1165, 1080, 1060, 1030 cm^{-1} to which a difinite assignment has not been made were apparent in all the spectra. Close resemblance of those spectra indicates that the vitreous, the tendon, and the substantia propria of the coenea are built from the protein of possibly a similar structure. This also provides support for the view that the protein of the vitreous belongs to the collagen type. (Table 1, Fig. 5)

Table I Interpretation of infrared spectra for vitreous, tendon, and substantia propria of cornea

Wave Numbers in cm^{-1}			Assignment
Vitreous	Cornea	Tendon	
3320	3320	3300	N-H strectceing vibration
3085	3085	3075	
2960	2955	2970	C-H stretching vibration
1655	1660	1660	C=O stretching vibration
1555	1550	1555	C-N stretching vibration+N-H in-plane deformation vibration
1455	1450	1455	C-H deformation vibration
1410	1405	1410	
1385	1385	1390	
1340	1335	1340	
1280	1275	1280	C-N stretching vibration+N-H in-plane deformation vibration
1240	1240	1240	
1210	1205	1210	
1165	1165	1165	
1080	1080	1080	
1060	1060	1060	
1030	1030	1030	
15μ Region	11μ Region	15μ Region	N-H out-plane deformation vibration

Discussion

In the present investigation, it has been proved that the potein of the vitreous belongs to the collagen type. On the other hand, the electron micrograph of the vitreous treated as described above, has revealed chiefly two types of fibrils. These two types of fibrils therefore must be responsible for those physical and chemical properties. In order to identify these two types of fibrils, analysis of each fraction must be carried out, but such an analysis would be difficult at the present stage. The patterns of the two fibrils however, must overlap in the result when a moderately large amount of materials are used. It may therefore, be reasonable to assume, that both types of the fibrils belong to the collagen type, in spite of great differences in the morphological features presented by the electron micrograph.

What, then, is the significance of the differences in the morphological features between these two types of fibrils and the usual collagen fiber, which is characterized by the axial periodicity of 640 Å and the width of about 1,000 Å?

In order to get information on the fibrogenesis at the developmental stage, the Achilles tendons of rat embryos, weighing from 2 to 4 g., were teased and examined by the electron microscope. The electron micrograph, as shown in Fig. 6, revealed a large number of fine fibrils, which were clearly striated and appeared like chains formed of fine granules. The axial periodicity varied between 540 Å and 930 Å, averaging 620 Å, and the fibril width ranged from 230 Å to 280 Å, averaging 250 Å. There is a good agreement between this fibril and B fibril of the vitreous. It was often observed that granules lay side by side, when two fibrils ran paralled in touch with each other. As the development progresses, a number of these fine fibrils decreases and the usual collagen fiber appears, gradually increasing in width. This indicates that the collagen fiber is produced by lateral aggregation of these fine fibrils. It may be assumed, therefore that B fibril of the vitreous is a primitive collagen fibril itself. (Fig. 6)

What then is A fibril of the vitreous? It is well known that some kind of collagen fiber dissolves in weak organic acid and is regenerated from such a solution, possessing axially repeating structures which are markedly influenced by the physical and chemical conditions of the regeneration. As the development progresses, changes may occur in the physical and chemical environments of the embryonic tissues, where active matabolism takes place. There is no reason for denying that A fibril is produced at some stage of development and B. at another. However, this is a matter of speculation, since fibrils such as A and B of the vitreous have not been produced experimentally. It may be safe, for the present, to say, that A fibril is also quite similar to a primitive collagen fibril. It seems reasonable, that the vitreous body, which must be in contact with the delicate tissue of the retina, is formed of primitive fine fibrils such as those of soft embryonic tissues. In order to explain the phenomenon of fibrogenesis by the theory of molecular structure, great efforts must be made, to produce experimentally these primitive fine fibrils and find the conditions which keep them stable. The vitreous body is the most suggestive for this purpose.

Summary

Electron microscopic, X-ray diffraction and infrared absorption studies of the vitreous of some kind of mammalian eyes were carried out. The results obtained were as follows:

1. X-ray analysis and infrared absorption data indicate that the proteins of the two types of fine fibrils which are presented by the electron micrograph of the vitreous belong to the collagen type.

2. The striated fibrils such as those of the vitreous are also found in the tendon of a rat embryo.

3. The striated fine fibril of the vitreous is a primitive collagen fibril, and the non-striated is similar to it.

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STUDIES ON ATP OF THE RETINA

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The recent knowledge about the role of phosphate plays in the muscular contraction, has enabled us to suppose, that the high energy phosphate compound with its easily releasable energy, may play a significant role in the chemical procedure of the vision.

Lange and Simon were among the first to show, that the isolated retina liberates more inorganic phosphate by illumination than by being kept in the dark, and when the illuminated retina was kept in the dark, this liberation of inorganic phosphate ceased more quickly by the use of a retina with pigmentepithelium than the one without pigmentepithelium. They concluded hypothetically that the liberation of inorganic phosphate from the retina caused by illumination was due to the breakdown of any organic phosphate in the retina.

Previous studies by Kuzuya and Tawara, searching for the source of liberated inorganic phosphate caused by illumination, resulted in the increase of inorganic phosphate and decrease in pyrophosphate, acid soluble organic phosphate and phosphagen.

By the use of iodometry and azotometry (Nakajima, Arata) and the use of histochemical techniques (Ozawa, Nakajima, Oguchi) the source of this inorganic phosphate was presumably believed to be phosphagen.

But recently Venkstern investigated the influence of light upon the phosphate fraction of the frog retina and reported that by illumination there were scarcely any changes in ATP and creatine phosphate, and that this fact would be due to the rapid resynthesis of these substances.

On the other hand, influence of light upon the respiration and glycolysis of the retina were studied by many workers on many animals but the results of these works were not in agreement with each other.

The purpose of this paper is to study the influence of light upon che metabolism of phosphate in the retina, especially that of ATP and creatine phosphate.

Phosphate distribution. After adequate dark (18 hours) or light (2 hours) adaption two sets of frogs were killed by decapitation in the dark or light room respectively. Retinae were removed rapidly from the eyeball and washed quickly with cold 0.6% NaCl solution. Adherent tissue and water were removed with filter paper, then placed

in a glass homogenizer, which contained 8.0 ml. of 10% trichloroacetic acid at 0°C. on cracked ice.

Distribution of phosphate was measured by the slightly modified LePage's and Fiske and Subbarow's method.

Light adaptation was made for 2 hours and during this period animals were placed in a white china box and illuminated by electric lamp. Special care was taken for the maintenance of temperature blow 10°C. In the fractionation procedure, in order to prevent the contamination by the inorganic phosphate liberated from the easily hydrolyzable phosphate, barium-insoluble fraction was made after inorganic phosphate had been removed. In colour developement, a neutralized sample was made acid with diluted H_2SO_4 and placed in a water-bath for 15 minutes at 25°C, then quickly measured by means of a spectrophotometer in the range of 700 Å.

It is the well known fact that the retina has a very high rate of respiration and glycolysis (aerobic and anaerobic). The energy which is amassed by this high rate of metabolism, may be stored in part in the high energy phosphate compounds as ATP and creatine phosphate, and both of them had been found in the retina. It is reasonable to suppose that the high energy phosphate compound may play a role in the chemical procedure of vision. As shown in tables 1 and 2, the value of Δ7P and creatine

TABLE 1

Phosphate distribution of the retina.

Experiment No.	Phosphate fraction			
	Δ7P	inorganic phosphate	acid-soluble phosphate	total phosphorous
Light adapted retina.				
1	6.58	21.90	249.60	1421.55
2	6.23	22.64	291.82	1180.47
3	6.04	21.52	315.13	1192.82
4	5.91	20.82	271.44	1153.63
5	6.23	21.53	339.52	1289.60
6	6.91	22.76	349.72	1343.62
7	7.29	23.85	318.75	1444.21
8	6.18	20.09	281.65	1303.98
Dark adapted retina.				
1	11.50	16.57	264.99	1031.70
2	10.75	15.32	254.51	1107.25
3	11.42	19.35	264.43	1105.52
4	11.16	18.62	258.05	1116.15
5	10.90	15.62	262.82	1113.74
6	11.28	19.78	257.19	1128.75
7	11.18	18.13	161.18	1120.75
8	11.82	19.36	267.41	1141.65

All figures are expressed as phosphorous γ/100 mg. dry weight of the retina.

TABLE 2

Phosphate distribution of the retina.

Experiment No.	Phosphate fraction			
	Cr-R	inorganic phosphate	acid-soluble phosphate	total phosphorous
Light adapted retina.				
1	42.89	23.36	229.93	1532.70
2	44.27	20.24	225.21	1520.24
3	42.58	20.67	224.05	1518.50
4	42.13	21.38	226.78	1422.00
5	43.54	22.17	223.96	1525.16
Dark adapted retina.				
1	57.17	16.97	221.29	1432.08
2	59.24	20.57	222.22	1450.03
3	59.81	17.13	228.03	1507.73
4	53.89	19.31	226.16	1485.52
5	54.73	18.52	227.31	1462.72

All figures are expressed as phosphorous /100 mg. dry weight of retina. Cr-P=creatine phosphate.

phosphate are higher in the dark adapted retina than in the light adapted one. This fact indicates the breakdown of ATP and creatine phosphate by light. The relative volume of ATP and creatine phosphate in the retina was in good agreement with that in muscle and brain, these facts lead to the conclusion that creatine phosphate may be the immediate phosphate compound which supplies phosphate to ADP to form ATP.

Venkstern and Sugita failed to find the change of ATP and creatine phosphate thru illumination, and said that no change in ATP and creatine phosphate is detectable after illuminatin, does not necessarily mean, that these compounds play no part in the chemical changes which is induced by light.

However, the finding of this change induced by light in ATP and creatine phosphate may be due:

1) to the use of a frog in winter while the animal has a very low metabolic rate and;

2) to the modification of fractionation procedure.

Determinrtion of ATP-ase activity. Determination of ATP-ase activity in retina was made, in vitro, to confirm the difference of breakdown of ATP between the light and dark condition.

Retinae were removed in the dark room, under dim red light after dark adaptation and placed in a glass homogenizer, containing 4.5 ml. of ice-cold 0.25 M sucrose, then homogenized in cracked ice.

Three test-tubes containing the same volume of reaction mixture were prepared. The reaction mixture always contained 1.0 ml. of homogenates, 0.5 ml. of 0.15 M KCl, 0.5 ml. of 0.2 M glycyl-glycine and 1.0 ml. of 10^{-3} M ATP (sodium salt). The substances were added in this order. ATP was added after the temperature of the mixture became

constant. For 0 time determination, all substances were added at a low temperature and ATP was added after the addition of ice-cold 20% trichloroacetic acid.

Two reaction mixtures were incubated for various periodes in a water-bath at 25°C, and during incubation one was illuminated and the other was kept in the dark. After incubation, cold trichloroacetic acid was added.

TABLE 3

Activity of the retinal adenosinetriphosphatase

Incubation time (minutes)	Experiment No.	illuminated	kept in the dark
5	1	35.93	20.51
	2	36.33	21.97
	3	37.93	19.83
	4	39.87	20.00
	5	40.00	20.25
15	1	46.60	47.88
	2	46.67	47.05
	3	41.89	42.24
	4	53.32	54.24
	5	39.12	39.25
40	1	54.65	55.17
	2	65.32	65.00
	3	62.40	67.21
	4	68.65	67.91
	5	60.61	60.58

All figures express the liberated inorganic phosphate as γ/10 mg. dry weight of the retina.

Inorganic phosphate of the deproteinized supernatant of three mixtures were measured by the method of Lowry and Lopez.

Table 3 shows the difference of the light and dark condition against the 0 time value. As seen in this table it is proved that the homogenate of the retina has the ability to liberate inorganic phosphate from ATP in both conditions. The value is two times greater in light condition than in the dark for the first 5 minutes, but the value of 15 and 30 minutes are equal in both conditions. The marked difference in the first 5 minutes may be accounted for the release of the last pyrophosphate of ATP by illumination.

Phosphate uptake. Influence of light upon the synthesis of organic phosphate from the inorganic phosphate was investigated in the retina by the use of inorganic isotope P^{32}, in vitro.

Retinae were removed from the dark or light adapted frogs. The homogenate was prepared with ice-cold 0.25 M sucrose by means of glass homogenizer and made pH 7.4 with diluted NaOH. The reaction mixture contanined 1.0 ml. of homogenate, 0.5 ml. of 0.2

M glycylglycine, 0.5 ml. of mixed solution of 0.15 M KCl and 0.006 M NaF, 1.0 ml. of mixed solution of KH_2PO_4 50 γ (as phosphorous) per ml. and inorganic isotope P^{32} 0.1 μc per ml. Incubation and deproteinization were made as described above. Extraction of inorganic phosphate from the reaction mixture was made according to Yoshizawa's isobuthanol extracting method. The specific activity of P^{32} which remained in the inorganic phosphate fraction of the reaction mixture, was measured by the use of

TABLE 4

Effect of NaF on the organic phosphate synthesis of the retina.

NaF added (final conc.)	Exp. No.	0 time	Kept in the dark	Illuminated
10^{-2} M	1	4567	4495	4461
〃	2	4525	4471	4431
〃	3	4496	4432	4393
10^{-3} M	1	4237	4051	4132
〃	2	4203	4015	4143
〃	3	4255	4039	4121

All values are expressed as $^1/_{10}$ c.p.m. per 5 mg dry weight of the retina.
Incubation 20 min., Temp. 25°C, pH at 7.4.

TABLE 5

Effect of pH on the organic phosphate synthesis of the retina.

pH of mixture	Exp. No.	0 time	Kept in the dark	Iluminated
6.8	1	4116	4041	4067
〃	2	4156	4089	4102
〃	3	4097	4011	4053
7.4	1	4121	3998	4051
〃	2	4217	4105	4162
〃	3	4139	4001	4057

All values are expressed as $^1/_{10}$ c.p.m. per 5 mg. dry weight of the retina.
In full mixture. Incubation 10 min., Temp. 25°C.

TABLE 6

Effect of temperature on the organic phosphate synthesis of the retina.

Temperature	Exp. No.	0 time	Kept in the dark	Illuminated
25°C	1	4112	3998	4053
〃	2	4161	4019	4079
〃	3	4138	4031	4087
30°C	1	4120	3918	3989
〃	2	4215	4001	4103
〃	3	4095	3888	3963
35°C	1	4116	3895	3973
〃	2	4182	3954	4041
〃	3	4091	3863	3957

All values are exprcssed as $^1/_{10}$ c.p.m. per 5 mg. dry weigkt of the retina.
In full mixture. Incubation 10 min., pH at 7.4.

Geiger-Müller counter.

Preliminary experiments were made to know the influence of concentration of NaF and pH and temperature upon the phosphate uptake of the reaction mixture. The influence of NaF was tested at the concentration of 10^{-3} M and 10^{-2} M. The phosphate uptake was greater in 10^{-3} M than in 10^{-2} M. When NaF was added to the reaction mixture at the concentration of 10^{-2}, phosphate uptake decreased markedly and this might be due to the inhibitory effect of NaF on tissue respiration rather than the effect of dephosphorylation (table 4). Effect of pH of the reaction mixture was checked at pH 6.8 and 7.4 and the phosphate uptake was greater at pH 7.4 than at 6.8 (table 5). The effect of temperature of the mixture was checked at 25°, 30° and 35° C. The phosphate uptake increased as the temperature of the mixture rose. But in the following experiments the temperature was always made to be 25°C, since the frog is a poikilothermic animal (table 6).

The results of the experiment on the phosphate uptake are shown in table 7. Decrement of specific activity in the inorganic phosphate fraction of the incubated reaction mixture against the 0 time value may indicate the formation of organic phosphate from the inorganic phosphate in the reaction mixture.

TABLE 7

Influence of light upon the organic peosphate synthesis of the retina.

Pigmentepithelium	Incubation min.	Exp. No.	0 time	Kept in the dark	Illuminated
without	10	1	4069	3890	3981
〃	〃	2	4131	4065	4111
〃	〃	3	4115	3942	3989
〃	〃	4	4023	3889	3926
〃	〃	5	4094	3803	3993
with	10	1	4125	3975	4081
〃	〃	2	4017	3777	3938
〃	〃	3	4165	4073	4106
〃	〃	4	4098	3944	4002
〃	〃	5	4064	3859	3908
without	20	1	4037	3896	3902
〃	〃	2	4114	4047	4094
〃	〃	3	4091	3885	3936
〃	〃	4	4003	3915	3945
〃	〃	5	4055	3839	3860
with	20	1	4044	3800	3979
〃	〃	2	4117	3809	3945
〃	〃	3	4035	3863	3902
〃	〃	4	4008	3845	3952
〃	〃	5	4109	3859	3967

All values are expressed as 1/10 c.p.m. per 5 mg. dry weight of the retina.
In full mixture. At pH 7.4, temp. 25°C.

So far as the experiment is concerned, the phosphate uptake by the retina, with no account of its pigmentepithelium, comes almost to the end in ten minutes, and in the next ten minutes much less phosphate uptake is observed, this can be only said for the case in which the retina was removed from the light adapted frogs. Results shown in table 7 suggest that the retina has ability to form organic phosphate from inorganic phosphate both in light and dark condition, and this ability is greater in the dark adapted retina than in the light adapted.

These facts may prove that the synthesis of organic phosphate from inorganic phosphate in the retina occurs as rapid as the breakdown of ATP and that pigmentepithelium has very little effect on the organic phosphate synthesis.

Effect of the addition of glucose, ATP, 2,4-dinitrophenol and monoiodoacetic acid on the phosphate uptake. Simillar experiments were made with addition of substrate, cofactor and inhibitor.

The reaction mixture was composed of 1) 1.0 ml. of homogenate (homogenized in the mixed solution of 0.25 M sucrose, 4×10^{-3} M NaF and 0.045 M KCl), 2) 0.5 ml. of mixed solution of 0.6 M glucose and 0.1 M $MgCl_2$, 3) 0.5 ml. of 0.5 M glicyl-glycine, 4) 1.0 ml. of mixed solution of KH_2PO_4 50 γ (as phosphorous) per ml. and inorganic phosphate P^{32} 0.1 μc per ml., 5) 1.0 ml. of water (or 10^{-3} M ATP, 4×10^{-5} M monoiodoacetic acid, and 4×10^{-4} M 2,4-dinitrophenol respectively for the porpose).

The same procedure as the above experiment was made and specific activity of P^{32} which remained in the inorganic phosphate fraction of the reaction mixture was measured.

TABLE 8 Effect of glucose.

Reaction mixture containes:
1) 1.0 ml. of homogenate (homogenized in the mixed solution of 0.25 M sucrose, 0.004 M NaF, and 0.045 M KCL). 2) 0.5 ml. of mixed solution of 0.6 M glucose and 0.1 M $MgCl_2$. 3) 0.5 ml. of 0.5 M glycyl-glycine. 4) 1.0 ml. of mixed solution of KH_2PO_4 50 r as P/ml. and P^{32} 0.1μc/ml. 5) Aq. distill. 1.0 ml.
Incubation time 20 min. pH 7.4, temp. 25°C.

Experiment No.	0 time	Kept in the dark	Illuminated
1	4532	4269	4369
2	4546	4259	4375
3	4517	4268	4379
4	4542	4290	4398
5	4540	4287	4404

All values are expressed as 1/10c. p.m. per 5 mg. dry weight of the retina.

TABLE 9 Effect of ATP

Reaction mixture containes:
1) 1.0 ml. of homogenate (homogenized in the mixed solution of 0.25 M sucrose, 0.004 M NaF, and 0.045 M KCL). 2) 0.5 ml of mixed solution of 0.6 M glucose and 0.1 M $MgCl_2$. 3) 0.5 ml. of 0.5 M glycyl-glycine. 4) 1.0 ml. of mixed solution of KH_2PO_4 50 r as P/ml. and P^{32} 0.1 μc/ml. 5) 1.0 ml. of 10 M^{-3} Na-ATP.
Incubation time 20 min pH 7.4, temp. 25°C.

Experiment No.	0 time	Kept in the dark	Illuminated
1	4548	4199	4341
2	4541	4199	4323
3	4591	4226	4372
4	4546	4212	4341
5	4542	4210	4327

All values are expressed as 1/10 c.p.m. per 5 mg. dry weight of the retina.

TABLE 10 Effect of 2,4-dinitrophenol

Reaction Mixture containes:
1) 1.0 ml. of homogenate (homogenized in the mixed solution of 0.25 M sucrose, 0.004 M NaF, and 0.045 M KCL). 2) 0.5 ml. of mixed solution of 0.6 M glucose and 0.1 M $MgCl_2$. 3) 0.5 ml. of 0.5 M glycyl-glycine. 4) 1.0 ml. of mixed solution of KH_2PO_4 50 r as p/ml. and P^{32} 0.1 μc/ml. 5) 1.0 ml. of 4×10^{-4} M 2,4-dinitrophenol.
Incubation time 20 min. pH 7.4, temp. 25°C.

Experment No.	0 time	Kept in the dark	Illuminated
1	4559	4538	4545
2	4561	4539	4543
3	4546	4505	4513
4	4586	4543	4559
5	4529	4480	4503

All values are expressed as 1/10 c.p.m. per 5 mg. dry weight of the retina.

TABLE 11 Effect of monoidoacetic Acid

Reaction mixture contains:
1) 1.0 ml. of homogenate (homogenized in the mixed solution of 0.25 M sucrose, 0.004 M NaF, and 0.045 M KCL). 2) 0.5 ml. of mixed solution of 0.6 M glucose and 0.1 M $MgCl_2$. 3) 0.5 ml. of 0.5 M glycyl-glycine. 4) 1.0 ml. of mixed solution of KH_2PO_4 50 r as P/ml. and P^{32} 0.1 μc/ml. 5) 1.0 ml. of 4×10^{-5} M monoiodoacetic acid.
Incubation time 20 min. pH 7.4, temp. 25°C.

Experiment No.	0 time	Kept in the dark	Illuminated
1	4546	4495	4531
2	4551	4493	4504
3	4571	4495	4529
4	4514	4451	4471
5	4497	4461	4455

All values are expressed as 1/10 c.p.m. per 5 mg. dry weight of the retina.

The results are shown in tables 8 to 11. By the addition of glucose and ATP, an increase in phosphate uptake was detected and this may indicate the acceleretion of glycolysis and respiration of the tissue by these substances. Monoiodoacetic acid and dinitrophenol inhibit the phosphate uptake of the tissue significantly, but the fact that the phosphate uptake is greater in the dark than in the light condition, remains unchanged.

These facts suggest that glucose is the fuel in the retina as well as in brain and the exsistence of some enzyme system which are inhibited by monoidoacetic acid and dinitrophenol.

Kerly and Bourne's works on glycolysis in the retina and the findings of aldolase and isomelase in the retina (Yoshizawa), cause us to suppose, that the early steps in retinal glycolysis may be as follow:

Sugars are phosphorylated to hexosediphosphate, which is phosphorylated to triose-phosphate, and oxydation of triosephosphate occurs with concurrent reduction of either a molecule of pyruvate or another molecule of triosephosphate and then occur the dehydration and dephosphorylation of phosphoglycerate to form pyruvate.

Glycolysis of the later steps are still obscure but the studies of the exsistence and activity of succinic dehydrogenase (Akiya and Nitta) and the findings of citric and malic acid in the retina (Krause and Stock) enable us to suppose the exsistence of some cycle like that of Krebs.

On the other hand Ochoa proved that high energy phosphate compound is formed in the brain by the same procedure as in the liver and kidney, and Palm reported, that in the rabbits anterior uvea the renewal of the organic phosphate largely takes place in the 7-minutes fraction.

Considering these facts mentioned above, it is possible to think, that the organic phosphate formed from inorganic phospnate by the retina, may be in part the high energy phosphate compound including ATP.

Very little is known about the utilization of energy stored in the high energy phosphate compounds, and studies about these aspects stimulate our interest.

Summary

The influence of light upon the phosphate metabolism of the retina was studied on the frog retina.

Distribution of phosphate in the retina was measured in vitro, in the light or dark adapted eye. The volume of ATP and creatinephosphate in the dark adapted retina was greater than in the light adapted one.

Influence of light upon the breakdown of ATP was tested in vitro. The breakdown of ATP occured more quickly in the illuminated retina than in the one kept in the dark.

Influence of light upon the synthesis of organic phosphate in the retina was investigated in vitro, by the use of isotope inorganic phosphate, and in the same time

influence of pH and temperature was checked. Then effect of the addition of glucose, ATP, 2,4-dinitrophenol and monoiodoacetic acid on phosphate uptake was tested. In all cases synthesis of organic phosphate occured both in the light and dark condition, but the volume of synthesized organic phosphate in the dark condition was greater than in the light.

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METABOLISM OF RIBOFLABIN IN THE BEHCET'S SYNDROME AND OTHER SIMILAR DISEASES

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Foreword

Behçet's syndrome was first described in 1937 by Behçet, but it has been left without much attention for a long time. Recently, this syndrome was mentioned in various fields such as in internal medicine, dermatology, otology, oral surgery and ophthalmology. These studies however done only on the comparison of the Behçet's syndrome Franceschetti-Valerio's syndrome, Stevens-Johnson's syndrome, Reiter's disease, Lipschuetz's acute vulvar ulcer, Neuman's aphthosis, Ectodermose erosive pluriorificielle, recurrent-hypopyon-iritis and chronic recurrent aphthosis. The basic studies of the Behçet's syndrome itself have not been undertaken although many theories have been introduced to explain the syndrome.

The present paper deals with the metabolism of riboflavin in Behçet's syndrome and other similar diseases.

Clinical studies. The authors investigated the pathological changes of the patients with Behçet's syndrome and found that the changes in the skin and mucous membrane, especially on the border line of these two tissues were similar to those of ariboflavinosis, and this fact has never been described by Behçet and other authors.

Thirteen patients with Behçet's syndrome were examined from April to September 1955 and the results are shown in Table 1. Following symptoms can be seen in the patients very frequently : in the eyes, pericorneal vascularization and infiltration, pannus,

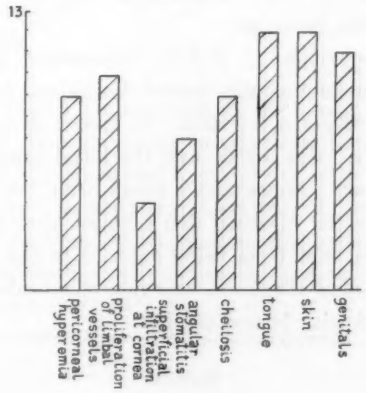
Table 1

Case	Years	Sex	Riboflavin in blood	Cornea	Mouth	Skin	Genitals
1	26	M.	10,57 (γ%)	+	+	−	+
2	48	M.	4,84	+	+	+	+
3	32	M.	5,69	+	+	+	+
4	34	M.	4,66	+	+	+	+
5	27	M.	12,15	+	+	+	+
6	59	F.	6,08	+	+	+	−
7	23	F.	8,98	+	+	+	+
8	27	M.	5,26	+	+	−	+
9	38	F.	6,99	+	+	+	+
10	31	M.	6,21	−	+	+	−
11	28	M.	5,60	−	+	+	+
12	30	M.	4,60	+	+	+	+
13	45	M.	2,62	+	+	+	+

and roughening of the corneal epithelium; in the mouth, fissure angular stomatitis on the angle of the mouth, glossitis, cheilosis; and pathological changes in the skin and genitalia; and so forth. These symptoms are different from the so-called muco-cutaneous-ocular syndrome and coincide with the changes of ariboflavinosis described by Sebrell, Spies, Youmans etc.

As shown in Fig. 1, three cases among these thirteen patients with Behçet's syndrome had all symptoms which are shown in Tab. 1, eight cases had more than

Figure 1



four symptoms and the rest had less than four symptoms.

Therefore, we can say that Beçet's syndrome shows the typical clinical symptoms of the ariboflavinosis.

Four patients who don't have aphthous stomatitis and genital ulcer but recurrent-hypopyon-iritis were examined and found to have symptoms of ariboflavinosis as shown in Tab. 2. They did not show, however, so many symptoms of ariboflavinosis as the patients with Behçet's syndrome.

Table 2

Case	Years	Sex	Riboflavin in blood	Cornea	Mouth	Skin	Genitals
1	43	M.	5,16 (γ%)	+	+	+	+
2	29	M.	2,88	+	+	−	−
3	20	F.	5,12	+	−	+	+
4	29	M.	5,85	+	−	+	−

Riboflavin metabolism in Behçet's syndrome We may conclude, therfore, that Behçet's syndrome is related to ariboflavinosis in some way, and that recurrent-hypopyon-iritis is related to Behçet's syndrome in this respect. The following experiments were done in order to clarify the riboflavin metabolism in these patients; (1) the determination of the total amount of riboflavin and (2) the bound form of riboflavin in blood and (3) the loading test of riboflavin, in which the riboflavin contents in blood and urine were estimated.

Method

Objects: Thirteen patients of Behçet's syndrome who visited our eye clinic from April to September 1955.

Sampling: venopuncture at the cubital vein was done with a 5cc syringe before breakfast anticoagulated with heparine.

Loading test: In cases 11 & 12, F.A.D. (equivalent to 20 mg. of free type of riboflavin) was injected subcutaneously before breakfast and the blood samples were taken before and 1 hour after the injection. In case 3 & 9, F.M.N. (equivalent to 10 mg. of free type riboflavin) was used, and the blood samples were taken before the injection and 30 min., 1 hour, 2 hours and 4 hours after the injection.

Patients urinated before the injection and 3 hours after the injection, the urine was taken. Then the urine was kept from being exposed to the light.

The method of determining riboflavin was microfluorophotometry of Fujita and the bound of it was determined with benzyl alcohol.

Results

Total riboflavin in the blood

Behçet's syndrome. Total riboflavin in the patients of Behçet's syndrome ranged from 2.62% to 10.57% as shown in Fig. 2. Average of these was 6.52% which was lower than 9.4% (± 1.09) of the normal persons as measured by one of the authors.

recurrent-hypopyon-iritis. The average of total riboflavin in the patients with recurrenthypopyon-iritis was 4.8% (ranging from 2.88% to 5.58%). It is lower than the average value in normal persons (Fig. 2).

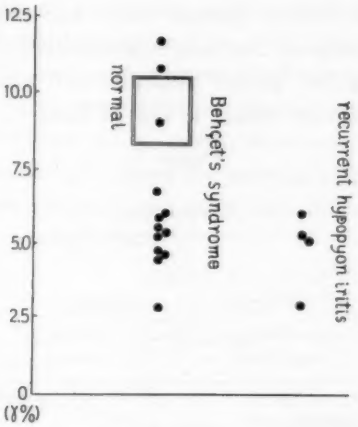


Figure 2 total riboflavin concentration in blood

The bound form of riboflavin in blood ester riboflavin ratio (Ester/Total×100) Loading test of riboflavin as to blood and urine.

Four cases among thirteen patients with Behçet's syndrome were examined.

Case 12 : 31 yrs. male

Clinical symptoms :

(1) recurrent-hypopyon-iritis

(2) aphthous stomatitis

(3) scrotal ulcer

Course : The patient noticed the decrease in the visual acquity of the left eye in July 1954. After that, the typical attack of recurrent-hypopyon-iritis and stomatitis ocurred about once a month. In September 1950, scrotal ulcer appeared, and disappeared after a while. Up to this time, antibiotics (streptomycin, penicillin, achromycin, aureomycin and teramycin) and cortisone were given to the patient without marked effect.

Bound type riboflavin in blood was found to be decreased markedly and ester ratio was only 13.0% (normal value is 90-100%).

In riboflavin load test a very low value was obtained, which is characteristic of ariboflavinosis. (Tab. 3)

Case 11 : 28 yrs. male

Clinical symptoms:

(1) recurrent-hypopyon-iritis

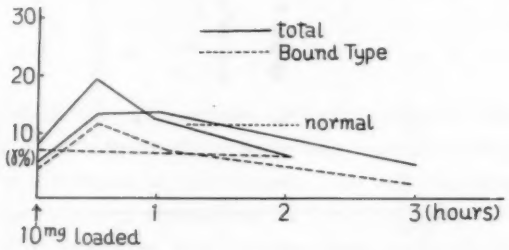
(2) aphthous stomatitis

(3) scrotal ulcer

(4) erythema nodosum

Course: Recurrent-hypopyon-iritis appeared suddenly in the left eye in Nov. 1953. Stomatitis and scrotal ulcer started in Nov. 1954. Erythema nodosum appeared in March 1955. Aphthous stomatitis, scrotal ulcer and erythema nodosum appeared repeatedly although an attack of the recurrent-hypopyon-iritis of the left eye did not occur very of ten. During the period, penicillin and vitamin K and C were given. The result of the evaluation was similar to that of Case 12. (Tab.4)

Figure 3



Riboflavin in blood (γ%)	total	ester	free.	ester ratio
before load	5,3	4,60	0,70	86,8%
after load				
30 m.	13,83	11,95	1,99	86,4
1 h.	14,32	8,56	5,76	59,8
2 h.				
3 h.	6,40	3,50	2,90	54,9
Riboflavin Excretion in Urine (mg)				
3 h.	4,59	quantity of urine 320 c.c.		

Case 3: 32 yrs, male

Clinical symptoms:

(1) recurrent-hypopyon-iritis

(2) aphthous stomatitis

(3) scrotal ulcer

(4) erythema nodosum

Corse: Recurrent-hypopyon-iritis in the left eye and erythema nodosum in the lower legs appeared in the Spring 1952. In October 1952, April 1953, October 1953 the recurrent-hypopyon-iritis of left eye appeared. In October 1953 and October 1954, the right eye also showed recurrent-hypopyon-iritis. Scrotal ulcer appeared in April 1955 and followed with several recurrence. No treatment was given during this period.

The result of evaluation was almost the same as in Case 12 and 11. (Fig. 3)

Case 9: 38 yrs. female

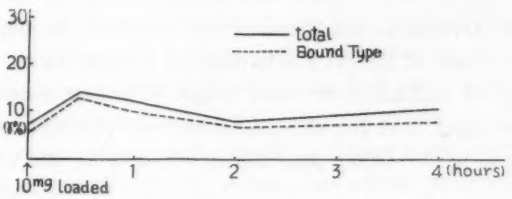
Clinical symptoms:

(1) recurrent-hypopyon-iritis
(2) apthous stomatitis
(3) acute vulvar ulcer
(4) erythema nodosum
(5) rheumatic arthritis

Course: In the Spring of 1948 acute vulvar ulcer appeared, and it reappeared about once a month. Stomatitis appeared in Feb. 1949, rheumatic arthritis in 1952, erythema nodosum in Oct. 1953 and recurrent-hypopyon-iritis of both eyes in Oct. 1954. These symptoms appeared very frequently. During this period, penicillin, cortisone, aminopyrin, etc. were used without effect, except, on the symptom of rheumatic arthritis.

The result of the evaluation was almost the same as in the preceding cases (Fig. 4). Total riboflavin, bound type riboflavin and ester ratio decreased in the blood of these four cases.

Figure 4



Riboflavin in blood (γ%)	total	ester	free	ester-ratio
before load	6,99			
after load				
30 m.	14,32	12,53	1,79	87,5%
1 h.	11,86	9,69	2,17	81,7
2 h.	7,65	6,76	2,11	78,4
4 h.	10,21	7,77	2,44	75,1
Riboflavin Excretion in Urine (mg)				
3 h.	4,556	quantity of urine 65 c.c.		

Riboflavin load test showed the characteristic feature of ariboflavinosis. In this test, riboflavin content in urine remained at the normal level in Case 11, Case 3 and Case 9. Only in Case 12 the value was increased to 17 mg. which was very high.

Discussion

The diagnosis of ariboflavinosis was discussed in the symposium held by the Japan Vitamin Society and Inoue's method was recognized to be good which includes judgemet from clinical symptoms, quantitative analysis of riboflavin in blood, loading test and clinical effects after continuous riboflavin administration. The typical symptoms of ariboflavinosis were found in most of Behçet's syndromes and recurrent-hypopyon-

iritis. They were seen much more frequently in these cases than in other chronic diseases like pulmonary tuberculosis and cirrhosis of the liver.

In our cases, the total riboflavin, the bound type riboflavin and ester ratio in blood were much lower than that of the normal values.

By loading test the increase in the amount of riboflavin after loading was very small. As the curves show, the transient increase immediately after loading, was small, and recovery of the previous value was prolonged.

The clinical effect after the continuous riboflavin administration are now being investigated and will be reported soon. However, some improvement of the symptoms have been seen already after the riboflavin administration.

So far, we can say that there is a disturbance in riboflavin metabolism in Behçet's syndrome and recurrent-hypopyon-iritis.

In this respect Schultheitz-Linder suggested the relation between this syndrome and vitamin B_2 complex in 1941. In 1954, Hotta reported that the administration of vitamin B_2 complex had improved these symptoms. But there are no literatures reporting the measurement of vitamin B_2 in these patients.

It is to be discussed, what causes the ariboflavinosis in the Behçet's syndrome. That is: whether the ariboflavinosis is one of the causes of Behçet's syndrome, or the ariboflavinosis is the result of Behçet's syndrome or ariboflavinosis is caused artifically by the administration of antibiotics or other drugs. The last possibility can be passed over because of the fact, that the ariboflavinosis was seen in four patients in whom no antibiotics had been used. Other possibilities are now on the way to be elucidated.

Table 3

Riboflavin Load Test (20 mg)	total	Bound Type	Free Type	Ester Ratio	Excretion in Urine 4 hours after load
before load	4,6(γ%)	0,6(γ%)	4,0(γ%)	13,0 (%)	
after load	5,8	0,6	5,2	10,3	17,2 mg
E.S.G.	1 hour			8 15	
Haemogram	Hb 80% erythrocytes 43×10 leucocytes 13,300 blood platelets 21,0×10			percentage of leucocyte neutrophilic 69,0 (%) eosinophilic 4,5 basophilic 0 lymphocytes 20,5 monocytes 5,0	
Function of Liver	total protein Bromsulphalein test Takada's reaction			7,8 7,5% (30′) (—)	
Function of Adrenal Gland	Thorn's test			a little decreased	
Wassermann's Reaction	(—)				

Table 4

Riboflavin Load Test (20 mg)	total	Bound Type	Free Type	Ester Ratio	Excretion in Urine 4 hours after load
before load	5,6(γ%)	3,4(γ%)	2,2(γ%)	50,7 (%)	
after load	8,2	4,2	4,0	51,2	5,2 mg
E.S.G.	1 hour			15 26	
Haemogram	Hb 10% erythrocytes 472×10 leucocytes 9,500 blood dlatelets 23,6×10			percntage of leucocyte neutrophilic 66,5% eosinophilic 9,0 basophilic 0 lymphocytes 21,0 monocytes 3,5	
Function of Liver	total protein Bromulphalein test Takada's reaction			8,0 7,5% (30′) (—)	
Function of Adrenal Gland	Thorn's test			a little decreased	
Wassermann's Reaction	(—)				

Conclusion and Summary

1. Thirteen cases of Behçet's syndrome and four cases of recurrent-hypopyon-iritis were examined in our clinic, and ariboflavinosis was found in all of these cases.

2. The total riboflavin, bound type riboflavin, and ratio in the blood are decreased in these cases. The loading test showed the characteristics of ariboflavinosis.

3. Decrease in ester ratio and the result of Thorn's test may suggest that there is some relation between the Behçet's syndrome and adreno-corticoal function.

4. Therefore we can conclude definitely that there is a disturbance in riboflavin metabolism in the Behçet's syndrome.

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ELECTROMYOGRAM OF THE PALSIED EXTRAOCULAR AND LEVATOR MUSCLES

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Introduction

Recently Björk, Kugelberg, and Kuboki succeeded to record the electromyogram of the human eye muscles and presented the detailed reports on its findings. But on the EMG findings on palsied eye muscles, there have been only Björk's descriptions, which reported about the EMG findings on 24 cases of the IIIrd. and IVth. cranial nerve palsies. In the present work, the electrical activities of the palsied eye muscles in 56 cases of the oculomotorius, trochlearis, and abducens nerve palsies were investigated and the characteristic alternations of the electrical activities were chiefly observed and discussed.

Method

Action currents were induced from four recti, two oblique, and the levator palpebrae superior muscles by a cocentric monopolar needle electrode and recorded by means of a cathode-ray oscilloscope with a suitable amplifier. Needle electrodes were made by shaving the piano wire with the file and varnishing it with cathew. A fine copper wire was soldered to the needle near the butt which was encased in plastic tubing. The indifferent electrode was inserted into the lobe of the ear. The needle electrode was inserted through the conjunctiva after local anaesthesia of 0.5 per cent techaine. To examine the levator palpebrae superioris, the needle electrode was inserted through the upper lid in the vicinity of the upper fornix.

Results

Reduction in the number of spike discharges. Reduction in the number of active units was generally recognized. This finding is caused by decreased innervation, due to destruction of motoneurones. But in clinically moderately pronounced pareses, it often happened that a considerable number of spike discharges were observed, and even among 8 cases of clinically complete nerve palsies, a few activations on interference voltage were observed in 6 cases, and no activation, e.g., "electrical silence" was ascertained only in 2 cases. Fig. I shows an example of this finding. Electromyogram is, in this meaning, clearly a useful diagnostic method of complete nerve palsy.

Low amplitude NMU Voltage (myogenic atrophy NMU Voltage). In 5 cases of palsied extraocular muscles, potentials of abnormally low amplitude, e.g., "myogenic atrophy-voltage", were recognized. The amplitude of spike discharges, which was observed in these palsied muscles, was from 70—80μV. to 20—30μV., that is, smaller than that of normal muscles, 200—300μV. The lowered amplitude and duration of action potentials

Figures

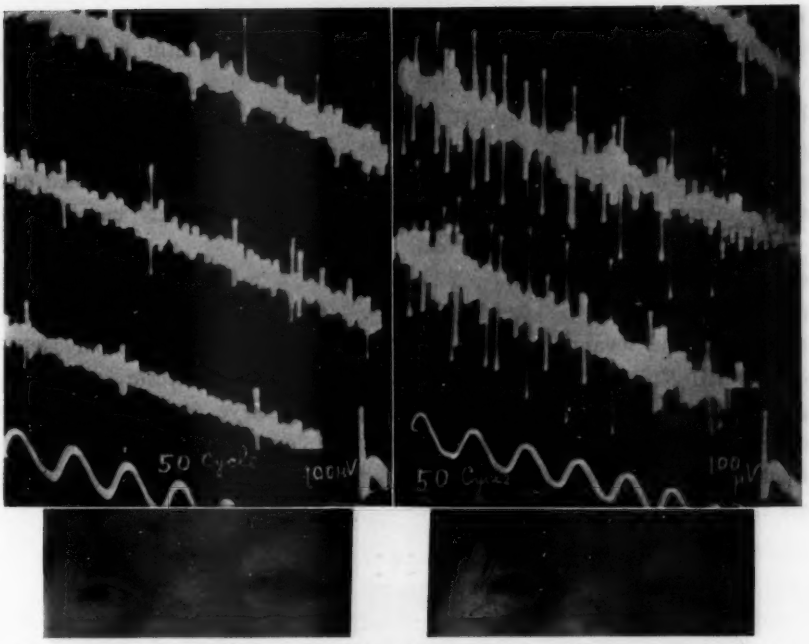

Fig. 1. Action currents from the right medial rectus muscle in a case of right oculomotorius nerve palsy (left) on gaze straight ahead (right) on gaze to the left

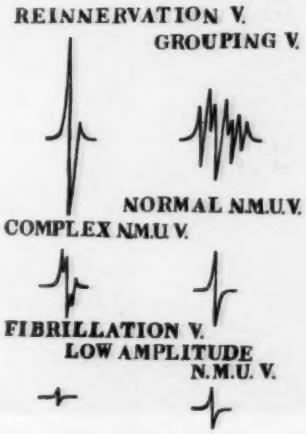

Fig. 2. Schema of abnormal NMU voltage (compared with normal NMU voltage) NMU. = Neuromuscular Unit

suggest the proceeding degeneration of muscle fiber parenchym and decrease of contractive function of muscle fibers despite of normal innervation.

Fibrillation Voltage. The fibrillation Voltage, which is a useful diagnostic finding of denervation in other skeletal muscles, has not been ascertained. Fibrillation Voltage is thought to be a spontaneous action potential of one or a few muscle fibers, beginning to degenerate in denervated muscles, so it is of very low voltage and its occurence is independent of the position and the movement of the eye. Fibrillations are difficult to recognize, parhaps, because the ocular muscles can not certainly be relaxed to the point where voluntary activity disappears.

Complex NMU Voltage. Polyphasic potentials, as recognized in Fig. 3 and 4, were observed in 11 cases among 43 cases of paretic extraocular muscles and 11 cases among 23 cases of paretic levator palpebrae muscles. The potentials of polycyclic shape, "complex NMU Voltage", is considered to appear in the paretic muscle in course of degeneration and regeneration of the peripheral nerve fibers when synchronized activation of muscle fibers belonging to one NMU is lost. The rarity of its appearance in the paretic extraocular muscles in comparison with that in other skeletal muscles suggests the lower innervation ratio of this muscle than other muscles.

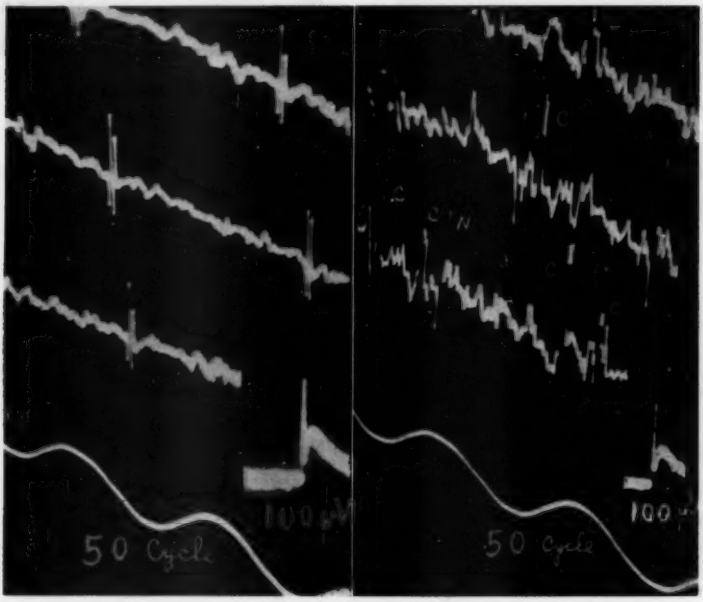

Fig. 3. Action currents from the medial rectus muscle in a case of right oculomotorius nerve palsy (complex NMU voltage)

Fig. 4. Action potentials from levator palpebrae sup. muscle in a case of oculomotorius nerve palsy (complex NMU voltage)

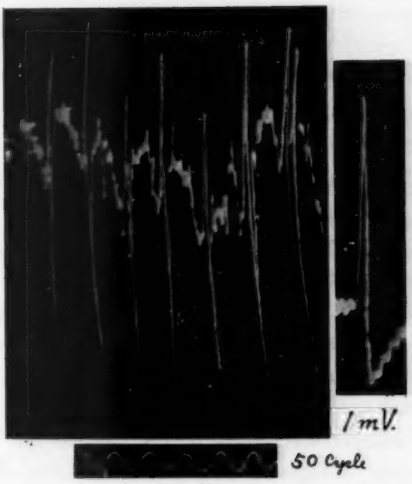

Fig. 5. Action current from the lateral rectus muscle in a case of abducens nerve palsy (Reinnervation voltage)

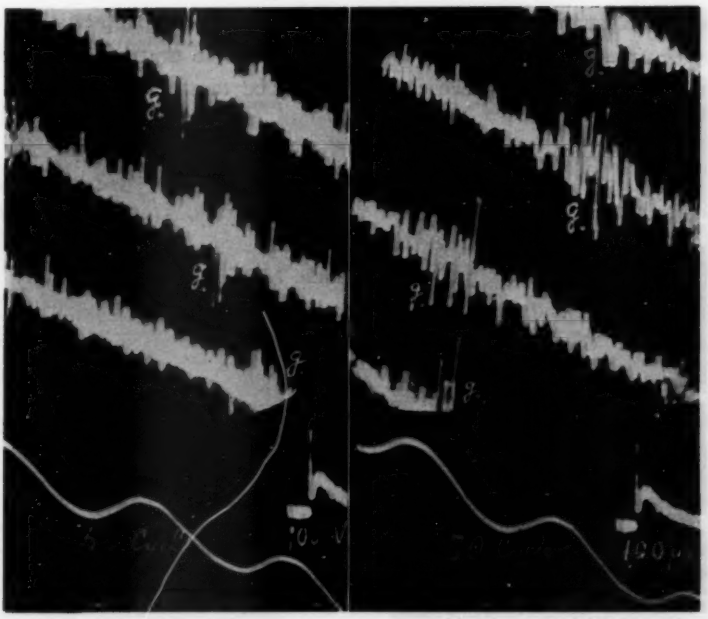

Fig. 6. Action potential from the right medial rectus muscle in a case of right oculomotorius nerve palsy (grouping voltage)

Reinnervation Voltage. High amplitude NMU Voltage, as high as 800μV. or 1 mV., was observed in 3 cases of paretic extraocular and 2 cases of paretic levator palpebrae muscles. This abnormal discharge, e.g., "Reinnervation Voltage", appears generally in paretic

muscles in course of regeneration of the peripheral nerve tracts, when a regenerated nerve fiber reinnervates more muscle fibers than before, that is why it has higher amplitude and longer duration than the normal NMU Voltage. An example of "Reinnervation Voltage" is shown in Fig. 5. Its appearance is of clinical importance suggesting, that reinnervation is proceeding in palsied muscles.

Grouping Voltage. The grouping Voltage, synchronized activation of a few NMU. (as seen in Fig. 6), or a tendency to grouping activation was observed in 3 cases of palsied extraocular and 8 of palpebrae muscles. Grouping activation is recognized in various cases on other skeletal muscles and many explanations can be given about their appearance on each case. The appearance of grouping voltage on muscles in fatigued, or cold-exposed, or de-sensory-innervated conditions, is explained as to be caused by decrease of proprioceptive impulses. No certain explanation can be given about the appearance of grouping voltage on eye muscles.

Characteristics of the interference voltage on the palsied eye muscles. The discharge density of the interference voltage on extraocular muscles was kept almost constant, so far as the visual line was fixed. But when a contractive effort was made in the palsied eye muscles, in cases of nerve palsy of high degree, uneven recruitement was often recognized. Uneven recruitement, a tendency to grouping activation and grouping voltage, may be caused by depression of facilitation of the central motoneuron, due to hypofunction of central motor nerve cells or proprioceptors.

Conclusion

Electromyographic investigations were made in the palsied extraocular and levator palpebrae superior muscles on 56 cases of oculomotorius, trochlearis, and abducens nerve palsies. Decrease in the number of spike discharges was generally ascertained. Abnormal discharges (Fig. 2), e.g., low amplitude NMU voltage, complex NMU voltage, Reinnervation voltage, and grouping voltage were demonstrated. Fibrillation voltage was not recognized. Characteristics of the interference voltage were discussed.

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FIBROUS STRUCTURE AND POLARIZATION OPTICAL PROPERTIES OF THE RABBIT CORNEA

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Previous workers have reported that the cornea exhibits a birefringence of uniaxial type. But as their observations were made in parallel polarized light only, it is desirable to make conoscopic observation as well in convergent polarized light. The general appearance of the cornea between crossed polars is such that the birefringent elements are predominantly arranged in a radial manner, although no anatomical evidence of such a radial arrangement has been reported.

Fibrous Structure

The excised corneas were sectioned, under freezing, tangentially or vertically to the surface into convenient thickness (100-120μ).

These sections were moderately dried at a room temperature, and mounted in Canada balsam to be observed under a polarizing microscope with crossed polars.

It was found by preliminary experiments that the presence of epithelial and endothelial layers hardly interferes with the birefringence of the stroma.

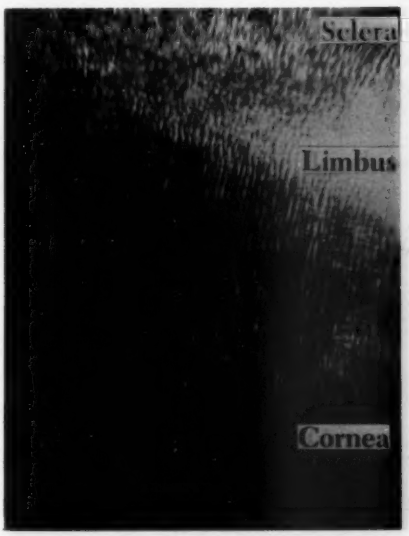

Fig. 1 Radially arranged fibers (Tangential section, peripheral region)

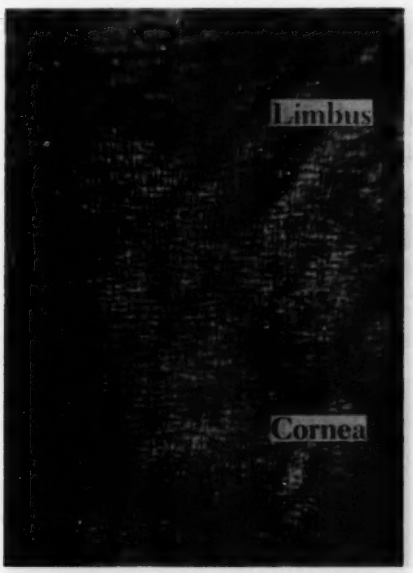

Fig. 2 An appearance of meshwork. The radial fibers are intersected closely with the other fibers. (Tangential section, peripheral region)

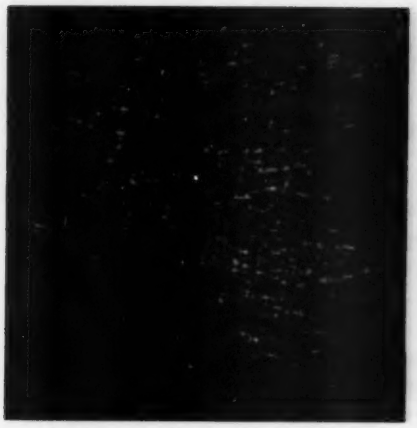

Fig. 3 An appearance of meshwork (Tangential section, central region)

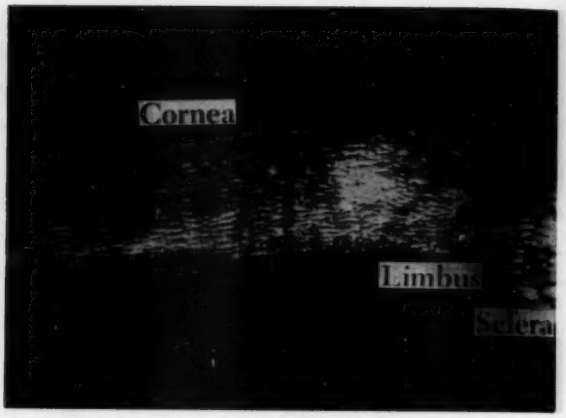

Fig. 4 A side view of the radially arranged fibers (Peripheral region, vertical section)

Fig. 5 Circular fibers. Arrow indicates the direction of the force.

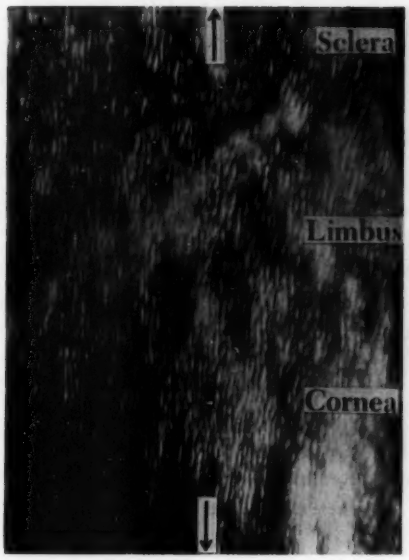

Fig. 6 Radial fibers. Arrow indicates the direction of the force.

The fibrous structure of the cornea which is revealed by the present investigation is the following.

Peripheral Region. In the posterior two-thirds of the cornea, the majority of the fibers are arranged in a radial manner, with a small number of intersecting fibers(Fig. 1).

On the other hand, in the anterior one-third, the radial fibers are intersected closely with the other fibers, forming an appearance of meshwork (Fig. 2).

Central Region. In all layers, the fibers intersect each other, resulting in an appearnce of meshowork (Fig. 3).

A side view of the cornea is shown in Fig. 4.

When stretched, it was found that the radial or circular fibers as the case may be, fibers which happened to lie parallel to the stretch, were selectively brought up in view (Figs. 5, and 6).

The average thickness of these fibers was found to be about 6μ by the use of an ocular micrometer.

Polarization Optical Properties

Isolated whole corneas were examined from the direction normal to the surface for the study of polarization optical properties.

Orthoscopic Observations

Peripheral Region. The radially arranged fibers were also observed between crossed polars in the whole (non-sectioned) corneas. These fibers were seen to the best advantage by turning the stage back and forth past the extinction position (the field of view was darkest), where the fiber direction was parallel with the plane of vibration of either polaroids. To ascertain the orientation of the vibration direction of the fibers, the stage (consequently the fiber direction) was turned 45° (diagonal position) from the position of extinction, either clockwise or counterclockwise, and a sensitive tint plate was inserted. When the fiber direction was parallel with the direction of vibration of the

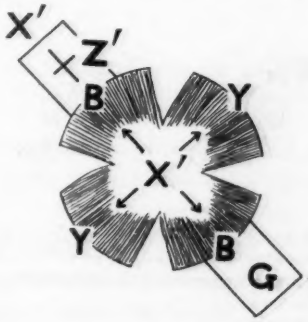

Fig. 7 Plane spread cornea between crossed polars in parallel polarized light with superposed gypsum plate. The fibers are arranged in a radial manner. X′ coincides with the fiber diretion. G— Gypsum plate, B—Blue (interference color), Y— Yellow (interference color).

fast ray (X′) in the sensitive tint plate, the interference color was raised (blue); when parallel with that of the slow ray (Z′), it was lowered (yellow). It is evident from these facts that the vibrational direction of the fast ray (X′) in the cornea coincides with the fiber direction, and that of the slow ray (Z′) is at right angles to the fibers (Fig. 7).

Central Region. The radially arranged fibers were observed to intersect crosswise at the central region. On rotating the stage, although those fibers which were in maximum illumination became dark, and those which were at the point of extinction became bright, the brightness of the field of view as a whole was not changed during the rotation of the stage.

Conoscopic Observations

Peripheral Region. An interference figure obtained at the periphery of the cornea was equivalent to that produced by a biaxial crystal plate cut perpendicularly to the acute bisectrix. The figure consisted of two isogyres, which together formed a dark cross, broad and poorly defined, in a certain stage position. On rotating the stage, the dark cross was divided into two hyperbolas in opposite quadrants; on further rotation, they formed a dark cross again. At a position of the stage, where they form a dark cross, the optic plane, including the optic axes, is parallel with the plane of vibration of either polaroids (Fig. 8, Ia, c). At 45° from the planes of the polaroids, the optic axes lie in the vertices of the two hyperbolas, which have their convex sides turned toward the acute bisectrix (Fig. 8, Ib, d).

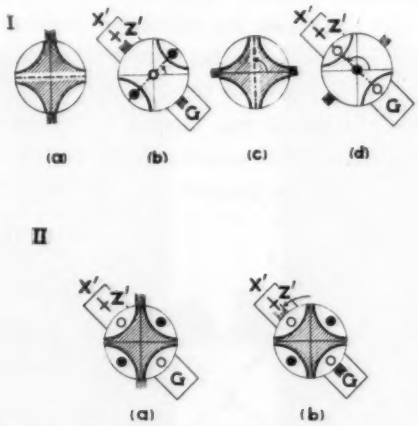

Fig. 8, I a-d. Biaxial acute bisectrix interference figure with superposed gypsum plate; rotation counter-clockwise, a—0°, b—45°, c—90°, d—135°.
II a-b Uniaxial interference figure with superposed gypsum plate.
○: A rise in interference color.
●: A fall in interference color.
Chain line—Z-axis, Optic plane.
Solid line—Vibration plane of polaroid.
The fibers seen under orthoscope are illustrated in the same figure. The Z-axis is at right angles to the fiber direction.

The acute bisectrix is normal to the plane of the cornea, and lies in the center of the field of view.

A sensitive tint plate was superposed over this interference figure with the direction of vibration of its fast ray (X′) parallel with the optic plane in the position at 45°, and the interference color on the concave sides was raised, while on the convex side it was lowered (Fig. 8, Id). When the slow ray (Z′) was parallel with the optic plane, all these were reversed (Fig. 8, Ib).

These figures indicate that the acute bisectrix coincides with the direction of vibration of the fastest ray, that is, the ellipsoid axis X. Therefore, the direction of of vibration of the slowest ray, the Z-axis, coincides with the line connecting the vertices of the two hyperbolas in the position at 45° (Fig. 8, Ib, d), and the Y-axis, that of the ray of intermediate velocity, is at right angles thereto. In this case, the sign is negative.

After the Z-axis had been located from an interference figure, the condenser and Bertrand lens were removed so that the fibers might be seen. Thus the Z-axis located from an interference figure was found to be at right angles to the fibers observed in parallel polarized light (Fig. 8, I).

The relations between a fiber and its ellipsoid axes are illustrated in Fig. 9.

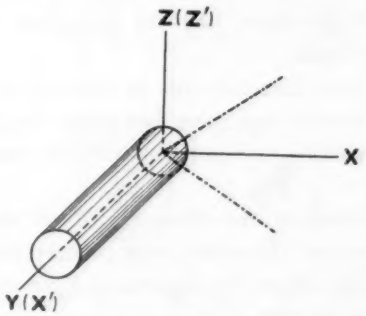

Fig. 9, Ellipsoid axes of a fiber. The acute bisectrix coincides with the X-axis, therefore, the sign is negative. The X-axis is normal to the surface of the cornea.

Central Region. An interference figure obtained in the central region of the cornea was a dark cross, broad and poorly defined, the arms of which were parallel with the planes of polaroids.

On rotating the stage, the dark cross exhibited no change; furthermore, lateral movement of the slide glass produced no change in the dark cross, so long as the central region occupied the field of view (Fig. 8, II).

This interference figure is equivalent to that produced by a uniaxial crystal plate cut perpendicularly to the optic axis; accordingly, the optic axis is normal to the plane of the cornea.

A sensitive tint plate being superposed over this interference figure, the dark cross was changed to a violet cross, The color between the arms fell to yellow in two quadrants, and rose to blue in the other two. The line connecting the centers of the blue quadrants made a negative sign with the direction of the fast ray in the sensitive tint plate (X′) as shown in Fig. 8, II, and the sign is negative.

The distance between the vertices of the hyperbolas at the 45° position in a biaxial interference figure varies with the size of the apparent optic angle. If the angle between the optic axes is supposed to diminish to 0°, the distance between the vertices of the two hyperbolas may diminish to zero, and a uniaxial interference figure may be produced.

Within a fiber some of the molecules must aggregate together to form micelles; these micelles are considered to be distributed in such a manner that the ellipsoid axes of all micelles are in parallel to each other.

At the central region, the radially arranged fibers were observed to intersect each other. This results in a random distribution of the micelles with regard to their Y and Z axes, but the X-axis will remain in the same direction. Hence, the refractive indices in the YZ plane are equal in all directions, and an apparent uniaxial interference figure may be produced under these conditions.

Rotation of the Z-axis by Stretching

A band-shaped strip was excised in such a way that its length was, as far as possible, parallel to that of the fibers, and the strip was examined between crossed polars in parallel polarized light.

With a sensitive tint plate inserted with its direction of the fast ray (X′) parallel to the fiber direction, the interference color was raised to blue. But when the strip was stretched in the fiber direction, the interference color changed from blue to yellow; namely it was lowered.

The direction of vibration of the slow ray (Z′) in the strip which was at right angles to the deforming force at the outset, was changed to the direction of the force by stretching (Fig. 10, I a,b). When the deforming force was removed, the interference color returned to blue again at once.

Another strip was excised so that its length was crosswise to the fibers, and the strip was examined between crossed polars in parallel polarized light.

With a sensitive tint plate inserted with its direction of the fast ray (X′) parallel to the fibers, namely at right angles to the length of the strip, the interference color was blue.

Then the strip was stretched at right angles to the fibers, but no remarkable change occured in interference color (Fig. 10, II a,b). In other words, when the deforming force was applied parallel to the direction of vibration of the slow ray (Z′), the original state was not disturbed by stretching.

Further, when these strips were examined in a convergent polarized light, a biaxial interference figure was obtained. The line connecting the vertices of the two hyperbolas at 45° position, the Z-axis, was at right angles to the fibers (Fig. 10, Ic, Fig. 10, IIc).

On stretching the strip in the fiber direction, the two hyperbolas approached the center of the field of view, formed a dark cross, and finally divided again into two hyperbolas, but in different quadrants (the Z-axis was not at right angles but in parallel to the fiber direction) (Fig. 10, Id, e). When the deforming force was removed, all these were reversed at once.

It is evident from the above facts that the Z-axis rotates about the X-axis to the direction of a deforming force, and returns to the original state when released.

But when stretched at right angles to the fibers, the two hyperbolas moved toward the edge of the field of view, eventually they disappeared, out of the field of view; in other words, there occured no rotation of the Z-axis, since the Z-axis was in the direction of the deforming force in the original state. Simply a widening of the optic angle takes place (Fig. 10, II d,e).

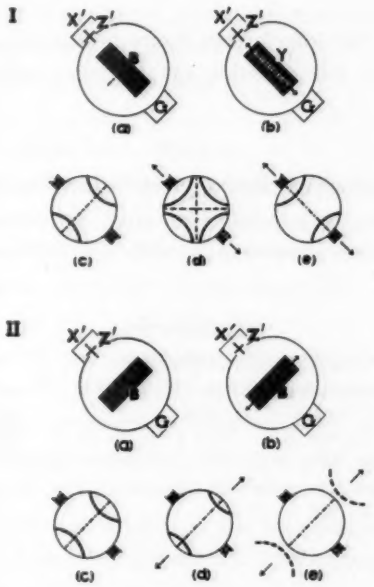

Fig. 10 Effects of stretching on the ellipsoid axes.
I a. A strip of cornea between crossed polars in parallel polarized light with superposed gypsum plate.
b. Ditto. Stretched parallel to the fiber direction.
c. The same strip between crossed polars in convergent polarized light. The fibers seen under orthoscope are illustrated in the same figure.
d,e. Ditto. Stretched parallel to the fiber direction.
IIa. A strip of the cornea between crossed polars in parallel polarized light with superposed gypsum plate.
b. Ditto. Stretceed at right angles to the fiber direction.
c. The same strip between crossed polars in convergent polarized light.
d,e. Ditto. Stretched at right angles to the fiber direction.
B—Blue (interference color), Y—Yellow (interference color),
Arrow—Direction of the force, Chain line—Z-axis.

It was confirmed, moreover, that the Z-axis rotates about the X-axis until it coincides with the direction of the deforming force, when the strip is stretched in a direction inclining by a certain angle to the fibers, and returns to the original state when released.

On the other hand, on stretching a strip of cornea, a remarkable cloudiness was observed, which, however, disappeared instantly when released.

Summary

The fiber arrangement was studied in detail.

The cornea is composed chiefly of radially arranged fibers.

The interference figure obtained at the periphery is biaxial; the Z-axis is at right angles to the fiber direction, the Y-axis is parallel thereto, and the X-axis is normal to the plane of the cornea; the X-axis coincides with the acute bisectrix, therefore, the sign is negative.

In the central region, the interference figure is uniaxial.

The Z-axis rotates to the direction of the force when stretched, but the X-axis remains unchanged.

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THERMOELASTICITY AND SUBMICROSCOPIC STRUCTURE OF THE RABBIT CORNEA

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In the present work, the fine structure of the cornea was analysed from the point of view of its mechanical behavior.

In the interpretation of the phenomena of rubber-like elasticity, the study of the changes in internal energy and entropy, which accompany an extension, is of particular

importance. These quantities may be derived from the temperature dependence of the elastic force or from the heat evolution on stretching.

Consider an object of length l acted on by an elastic force K. In a small displacement dl, the work done on the object is Kdl. Assuming constancy of temperature, this work is, by definition, equal to the change dA in the Helmholtz free energy, i.e.

$$K=(\partial A/\partial l)_T. \cdots\cdots(1)$$

This may be written in the equivalent form,

$$K=(\partial E/\partial l)_T-T(\partial S/\partial l)_T, \cdots\cdots(2)$$

where E is the internal energy and S the entropy.

The entropy of extension at constant temperature may be related to observable quantities by the following equation:

$$(\partial S/\partial l)_T=-(\partial K/\partial T)_l \cdots\cdots(3)$$

Over a small range of temperature, K may be treated as a linear function of T (the curve $K=f(T)$ is replaced by its tangent).

The equation of this straight line is then,

$$K=a+bT, \cdots\cdots(4)$$

where $a=(\partial E/\partial l)_T$ and $b=(\partial K/\partial T)_l=-(\partial S/\partial l)_T$.

In terms of the mechanical theory of heat, $(\partial E/\partial l)_T$ represents the change in the potential energy of molecules or parts of molecules, which takes place during an isothermal change in length of an object. Moreover, the entropy of the system is related to the thermodynamic probability by the equation

$$S=R\ ln\ P.$$

P provides a measure of the possibilities of movement and rearrangement open to the kinetic units. If, therefore, the entropy (S) increases on stretching the object, this means that deformation provokes a transformation from a more ordered (less probable) to a less ordered (more probable) condition—the sphere of movement of the kinetic units is increased.

Conversely, if S diminishes on stretching, the possibilities of rearrangement and of movement of the units are limited by the stretch—the system becomes more orderly

Method

To determine the temperature coefficient of the elastic force exerted by a piece of cornea stretched at constant elongation, the dynamometer of Polanyi was used here (Fig. 1). It was possible to measure tension change under practically isometric conditions with an accuracy of ± 0.01g.

A strip of the cornea of 4 mm width was excised, and both ends were tied with cotton threads to a pair of glass shafts.

Thereupon the preparation was mounted to the apparatus: the upper shaft hung on a steel spring of the apparatus and the lower was firmly screwed to a support,

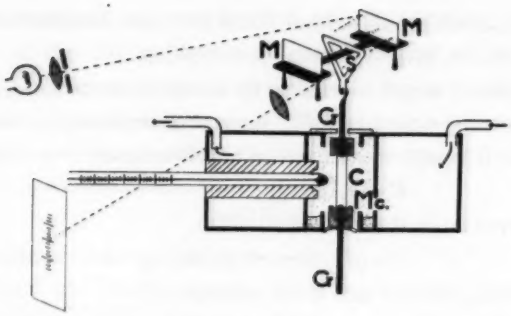

Fig. 1. Dynamometer of Polanyi.
M—Mirror, S—Steel spring, G—Glass shaft, C—Cornea,
Mc—Moist chamber.

which was adjustable in the vertical direction with a fine pitch screw. The preparation was kept in a moist chamber, as protection against swelling or shrinkage, and the temperature of the chamber was changed by passing heated or cooled water through a bath, in which the chamber had been installed. The temperature of the chamber could be changed from 15° to 30°C. in about 10 min.

Results

Preceding to the experiment on the tension-temperature relations, a preparation was stretched and kept at a constant length at 30°C, during which the tension fell gradually, and eventually became constant; thus an equilibrium was reached. This steady value is termed initial tension.

Tension-temperature Relations when Stretched in the Fiber Direction. A strip was excised so that the cornea could be stretched in the fiber direction (Fig. 2). After a state of equilibrium was reached, the temperature was changed. Fig. 3 shows the effects of a change in temperature on the force exerted by a strip of the cornea at intermediate

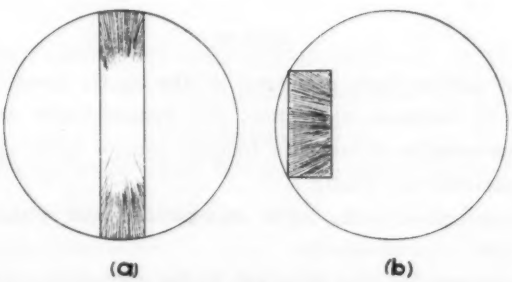

Fig. 2. Two modes of the excision of a strip from the cornea.

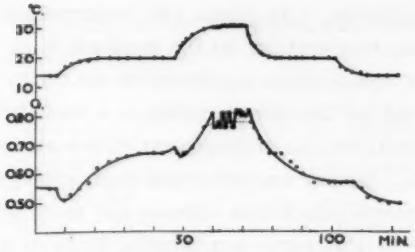

Fig. 3. The effect of a change in temperature on the force exerted by a strip of the cornea. The figure shows simultaneously the change in temperature and the change in elastic force with time. There is a tendency for the tension to fluctuate at 30°C.

elongation. The tension increases gradually by raising the temperature, eventually a steady value is obtained, i.e. the second equilibrium is reached, and these are reversed on cooling. But at 30°C. there is a tendency for the tension to fluctuate, so the mean value is taken as the tension. The tension-temperature relations at different elongations are summarized in Fig. 4. Since the initial and final values for the elastic force do not coincide, in general, the tangents are drawn to the upper portions of the ascending limbs to represent the force.

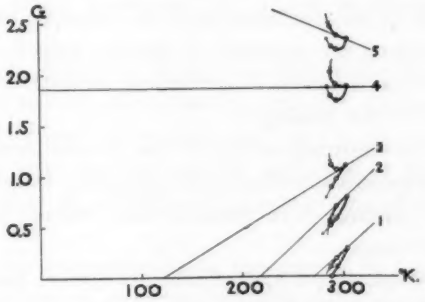

Fig. 4. Curves showing the elastic force exerted by different strips stretched parallel to the fiber direction at various elongations, plotted as a function of the absolute temperature.

The elastic force increases with rising temperature at slight or intermediate elongations, the temperature coefficient of the elastic force at constant length being positive, but it decreases for greater elongations, a negative coefficient being obtained.

Tension-temperature Relations, when Stretched at Right Angles to the Fibers.

A strip was excised so that the cornea could be stretched at right angles to the fiber direction (Fig. 2). The tension-temperature relations were entirely similar to that described above.

The Molecular Interpretation. At slight and intermediate elongations, the elastic force increases with rising temperature, so the constant b in equation (4) is positive, and as $b=-(\partial S/\partial l)_T$; the temperature coefficient of the elastic force is positive. Therefore, isothermal stretching of the cornea produces a diminution in the entropy (S) of the system (dS is negative), i.e. the arrangement of the atoms or groups becomes more ordered and less probable. During an isothermal contraction, the entropy S increases, i.e. the ordered system passes into a less ordered and more probable condition.

The tangent drawn to the curve representing force as a function of the absolute temperature makes a negative intercept on the K-axis, i.e. a in equation (4) is negative, and $a=(\partial E/\partial l)_T$. Therefore, the internal energy (E) diminishes during isothermal stretching (dE is negative); it follows that the decrease of TS must be greater than the increase of the free energy A, since $dE=dA+TdS$. As the heat given off equals TdS, this heat is greater than dA, the work absorbed.

In brief, during an isothermal stretch, the tendency for a more ordered arrangement of the kinetic units to supervene is so great that more heat is given off than is absorbed as work; this means that during an isothermal stretch the increase in orientation proceeds with evolution of latent heat. In other words, a sort of crystallization takes place as a result of stretching; on allowing the cornea to contract, dE becomes positive, so that the "crystallites" melt with disorientation of the ordered system.

Such disorientation is accompanied by a macroscopic contraction of the stretched cornea. This fact leads to the conclusion that the atoms or groups of atoms are bound together by strong bonds in the direction of stretch, namely the cornea is built up of chain molecules. The rearrangement on stretching consists, therefore, in straightening and parallel orientation of the chains.

The elastic force of a deformed cornea is due to the thermal motion of segments of long, flexible, chain molecules stretched by deformation. On releasing, the straightened chains tend to return, through free rotation about valence bonds, to any statistically more probable (twisted) form.

Furthermore, the cornea shows limited relaxation when held stretched at a constant length, i.e. the tension falls to a constant level above zero. This indicates that the chains are united by chemical bonds to form a loose network.

Submicroscopic Structure. It has been shown by studies with polarized light that the cornea is composed of radially arranged fibers, the Z-axis lies at right angles to the fiber direction, and the Z-axis rotates about the X-axis in the direction of a deforming force on stretching. Moreover, from the thermodynamic analysis, it has been concluded that the cornea is built up of loose networks of flexible long chain molecules.

On stretching such a network parallel to the fiber direction, the chains are straightened and orientated, and the arrangement of atoms or groups of atoms becomes more orderly, and the micelles are caused to rotate in the direction of the force (Fig. 5, I). The behavior of the network, when stretched at right angles to the fiber

direction, is essentially the same as that described above except that there occurs no rotation of the micelles (Fig. 5, II).

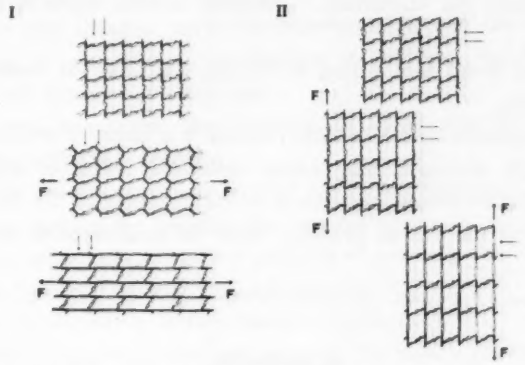

Fig. 5, I. Straightening and parallel orientation of the chains with rotation of the Z-axis when stretched parallel to the fiber direction.
II. Straightening and parallel orientation of the chains with no rotation of the Z-axis when stretched at right angles to the fiber direction.

The cornea may be assumed to be built up of many layers of a two-dimensional network. A remarkable increase in thickness on swelling may well be explained on the assumption that these layers are not united by strong chemical bonds with each other.

The cornea, however, dose not expand in other directions on swelling, since the the chain molecules in a network are united by stronger chemical bonds.

The temporary impairment of transparency, due to an excess pressure applied to the cornea, is believed to be caused not only by imbibition of fluid, but also by physical changes in the stroma, since the cloudiness disappears immediately when the pressure is lowered.

On the other hand, the transparency of the cornea is explained by assuming a uniform change in the index of refraction in each fibers, as well as from the fiber to the surrounding medium. In the present work, it was shown that a sort of crystallization is accompanied by reversible cloudiness in stretching.

In this case, the reversible cloudiness may be considered as due to an abrupt difference in refractive index between the "crystallites" and the surrounding medium.

Summary

The effect of temperature on the elastic force of a stretched cornea at a constant length has been investigated.

At slight and intermediate elongations, the temperature-coefficient of the elastic force is positive, and the tangent draw to the curve representing force as a function of the absolute temperature, makes a negative interception on the K (force)-axis.

From these data, it may be concluded:

(a) The cornea consists of flexible, long chain molecules, the arrangement of which becomes more ordered on stretching. Thermal motion tends to provoke a return to the original state;

(b) Latent heat is set free during stretching and may be considered as a sort of heat of crystallization.

These results indicate that a corneal lamella is a sheet of two-dimensional network, composed of radially arranged long chain molecules, some of which are aggregated together to form micelles, whose long axis is at right angles to the fiber. The reversible cloudiness caused by an excess pressure may be considered as due to a sort of crystallization.

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AN EMBRYOLOGICAL STUDY ON THE HUMAN CORNEAL NERVES

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Introduction

Morphological studies on the human corneal nerves have been reported in large numbers, while on embryological observations extremely few.

In 1896, Bach reported on the newborn's corneal nerves, dyed by Golgi-Cajal's osmiumbichromat silver staining, that about 30 nerve-trunks went into the cornea from the limbus, dichotomising, and becoming delicate gradually. Their branches, which ran irregularly but showed a certain type, formed a so-called "Network" with free endings.

Lately the process of the embryonal development of the human corneal nerves have been clarified systematically.

Meterial and Method

For experimental material, 64 Japanese human embryos from one to ten months old were used. And for reference, four corneas of adults, too.

Among various kinds of methods of staining the peripheral nerve fibre, the eight

were chosen ones, thought to be typical, that is, those by Bielschowsky, Boeke, Gros-Schultze, Fujita, Agduhr, Cajal, Bodian, and Fujimori-Narita.

Having been applied each one of them to the human embryo's corneal nerves, they were compared, one with another, as to the representability of the nerve fibre and the degree of co-staining of other tissue, the stability of staining, the degree of shrinking in the tissue, and the easiness of technique.

From this, it was concluded, that method by Boeke was the most adequate and excellent one for the following reasons.

1) Distinctly the human embryo's corneal nerve is represented by it.

2) It has an inclination for staining the corneal tissue to some extent, however, it is superior to any other method in the stability of staining the nerve tissue.

3) It permits serial section and the technique is easy.

4) The degree of shrinking in the tissue is medium.

5) No restriction is given by the speciality of the object, the human embryo.

6) There is little artifical production.

The corneas stained by this method, further, methyleneblue, H-E, Azan's, Weigert's, Loewit's staining etc, were sectioned serially in 5~20μ thick horizontally, vertically, and obliquelly.

Results

As to the one-month-old embryos, no nervous elements are observed in the mesodermal cell group surrounding the optic cup. But at the middle of the second month, two or three nervi ciliares are recognized piercing obliquelly the primary sclera at the back of the optic vesicle, and at the end of this stage, they reach the edge of the optic cup, where they acquire some fine branches as origin of Plexus ciliares.

In the third month, the nervi ciliares, which have come directly through the deeper stratum of the primary sclera make their way, for the first time, through the substantia propria as corneal nerves.

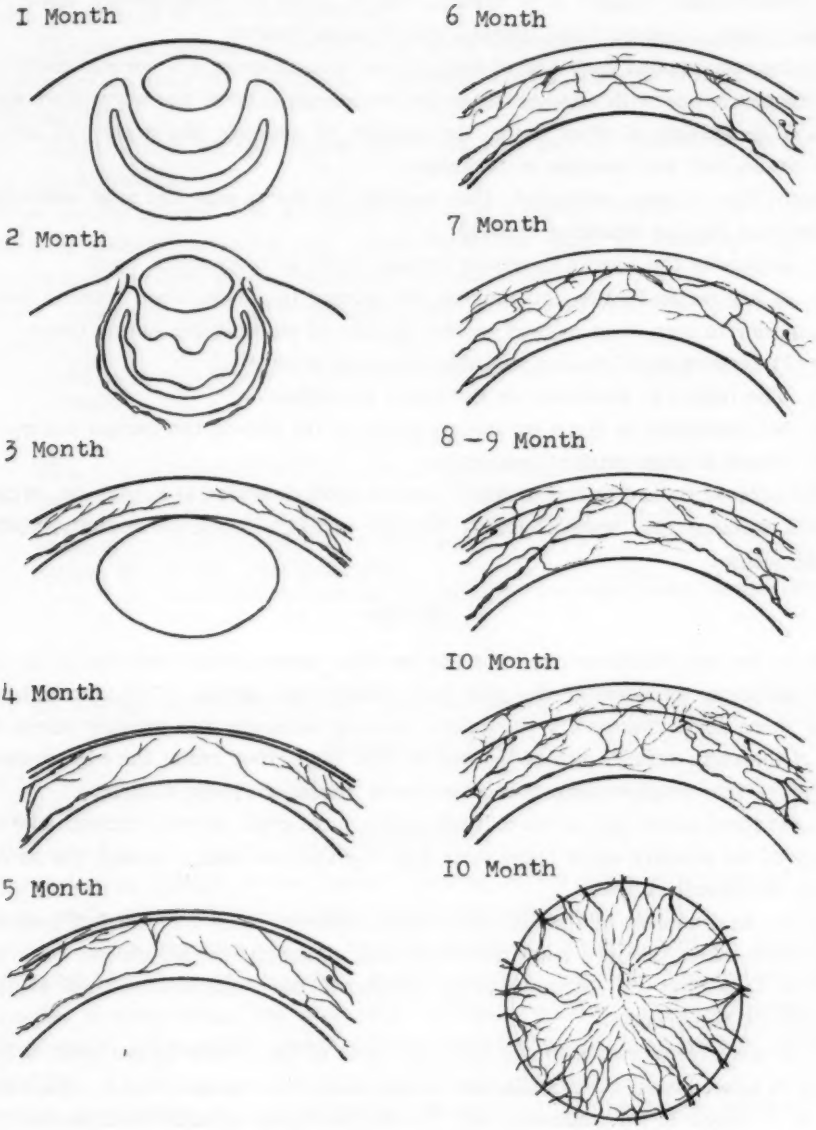
In the environment of the cornea, Plexus annulares being composed of conjunctival nerve fibres which advance from the fornix and superficial scleral nerves.

It is then that the cornea having completed the endothelium, just begins the fibrilation of the propria.

From the second month to the third, the base of the nerves in the orbit is formed, namely N. nasociliares, N. frontalis, and N. lacrimalis differentiate from N. ophthalmicus, and N. trochlearis, N. abducens and N. oculomotorius advance each toward its own muscle in order to innervate it.

Concerning the skin, nerves develope in the corium. In other words, this may be considered as the important period during which innervated nerves reach the peripheral organs, just beginning the differentiation.

In the last stage of the third month, two kinds of nerve fibres entering the cornea



Embryonal Development of Human Corneal Nerves (Schema)

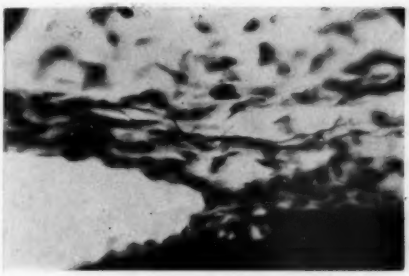


Fig. 1 3 month; corneal nerves advance into deep substantia propria.

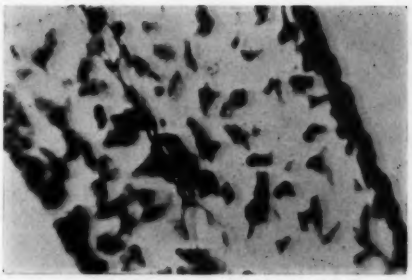


Fig. 2 3 month; Typical acute angled dichotomie.

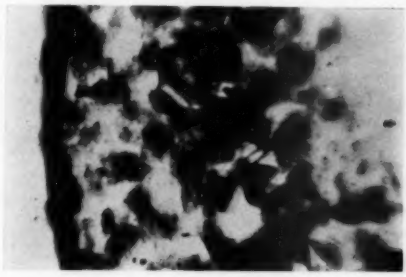


Fig. 3 4 month; Obtuse angled dichotomie

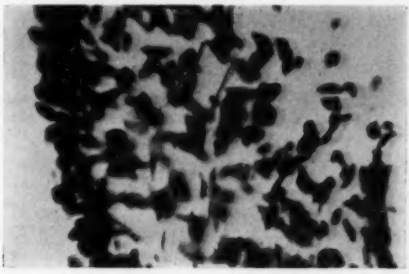


Fig. 4 5 month; Nerves to go into propria are devided at limbus into three steps.

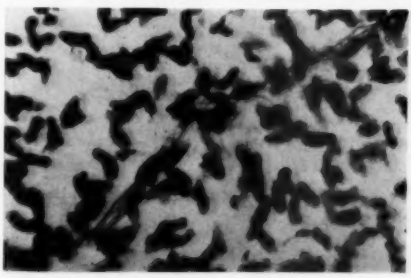


Fig. 5 6 month; Fine branches go towards epithelium.

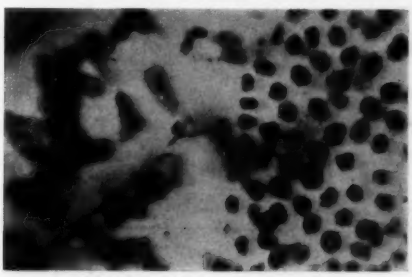


Fig. 6 6 month; Nerve fibre makes way through in one to epithelium.

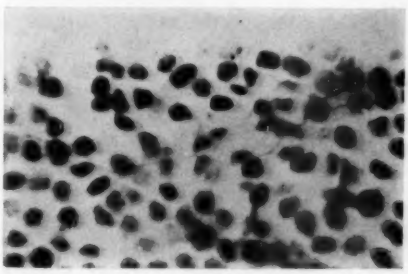


Fig. 7 6 month; Distribution in epithelium.

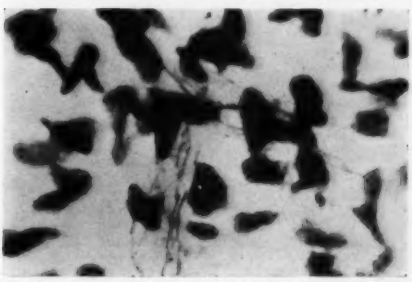


Fig. 8 7 month; Terminalreticulum (Stoehr).

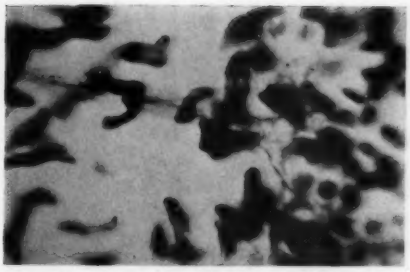


Fig. 9 7 month; Branching off below epithelium.

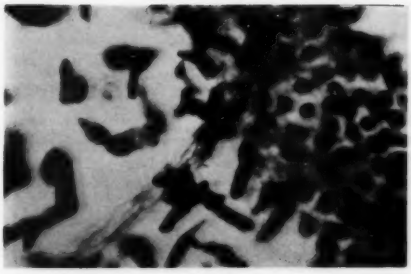


Fig. 10 7 month; Go into epithelium twining round each other.

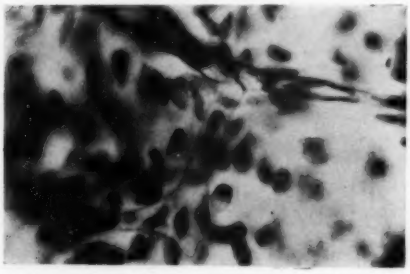


Fig. 11 8 month; Association as chiasm at limbus.

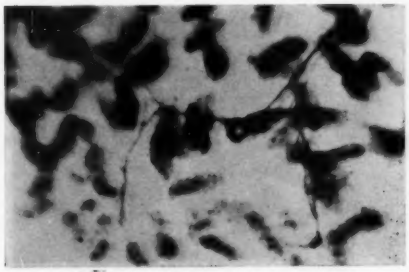


Fig. 12 8 month; Atypical ramification

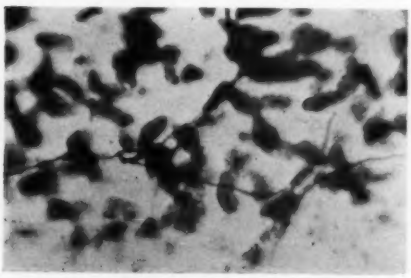


Fig. 13 8 montn; Atypical ramification

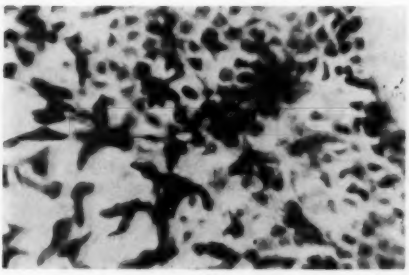


Fig. 14 9 month; Branching off in epithelium

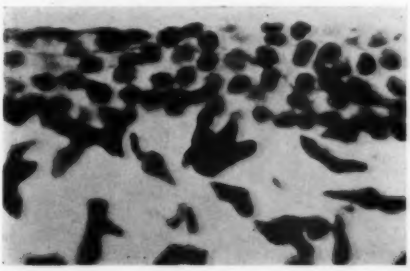


Fig. 15 9 month; Transversal fibres in epithelium

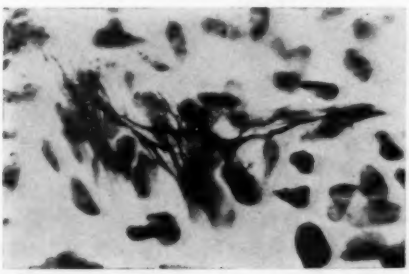


Fig. 16 10 month; Strong trunks Attias I

are recognized which are bundles of thick nerves running from the deeper stratum of the propria to the center region of the cornea (Fig. 1), making typical acute angled dichotomies (Fig. 2), and fine ones which distribute at the peripheral cornea from the superior stratum.

In 85 embryo's cornea there are 5~8 bundles of nerve fibres which go into the cornea vertically to the limbus.

In four month, the form of the ramification becomes complicated and irregular. The deeper nerve bundles have acute angled dichotomies at the limbus, but in the center obtused angled ones from 1 to 3 times ascending through the propria (Fig. 3), and their branches wave up to the right below the Bowman's membrane.

At the fifth month, they, penetrating the Bowman's membrane distribute themselves in the epithelium in the state of so-called "rami perforans".

As to the skin, appearance of nerve fibres in the epidermis is from the end of the fourth month to the fifth. It is of much interest, that the development of the nerves in the skin are in parallel with that of the corneal nerves.

The nerves to go into the propria, are devided at the limbus into three—upper, middle and a deeper strata (Fig. 5).

Delicate ones which go through the upper stratum are distributed along the edge of the cornea, and middle-sized or large nerve trunks, which go through the back are distributed in the center region.

The nerve trunks entering into the cornea vertically to the limbus, do not always run to the center, some turn back half-way, drawing an arc as "rami rucurrence", or run parallel to the limbus.

In the sixth month, nerve fibres for the epithelium make their way through 1) in one (Fig. 6), 2) four or five twining rounds, 3) branching off, and over the epithelium their fine branches are scattered, drawing an arc, turning back, zigzagging, and anastomosing each other (Fig. 7).

In the seventh month spreading of associational fibres among the nerve trunks is noticeable. Their region is sometimes broadened to more than 150μ.

The general growth of the cornea has been completed, corneal nerves remarkably develop towards the formation of plexus substantia propria corneae. Besides typical dichotomies, various kinds of atypic ramifications are recognized, especially in the pupillar region. (Fig. 12, 13.).

The nerve ending has a "terminalreticulum" by Stoehr (Fig. 8), in addition to the general nature of free-ending.

In the eighth month to the tenth, the corneal nerves are, according to the size and the state of entering the cornea, clearly devided into four types of Attias's (Fig. 16).

In the cornea their ramification and anastomosis increase to make a solid nerve-net. Thus they come to organize Plexus paramarginalis superficialis, Plexus substantia propria corneae, Plexus subepithelialis, Plexus intraepithelialis, as recognized in the adult. The

nervous elements are limitted almost to two-thirds of the front of the cornea, and the more closely towards the surface.

The mode of intraepithelial nerve fibres can be classified into four types, such as 1) branching off radiately (Fig. 14), 2) running transversally parallel to the besal stratum (Fig. 15), sometimes they penetrate again the Bowman's membrane, to come back to the propria, 3) run straight with few ramification, 4) as a network like terminalreticulum.

In the tenth month, the nerve bundles into the cornea from Plexus annularis amount to 35~40.

The nerve endings in the cornea are devided as follows:

1) free ending that is ubiquitous throughout embryo and adult.

2) thickend tip like a club, may be characteristic of embryonal nerves.

3) retiform ending, probably the same as the terminalreticulum by Stoher.

4) blob with a clear halo about the size of $1.0\times1.5\mu$, recognized as only a few at the superficial stratum of the peripheral propria.

There are no specifical end-bodies as an "Endknoepfchen" in the epithelium.

Summary

Having applied Boeke's silver-staining to corneas of 64 Japanese embryos of one-month to ten-month old, the corneal nerves were observed. Here follows the record of the observations.

1. one-month-old embryo.

The cornea is not yet taking shape and no nervous elements are found in the orbit.

2. two-month-old embryo.

While the primary cornea is being formed, the nervi ciliares reach the edge of the optic cup.

3. three-month-old embryo.

Fine nerves begin to appear in the substantia propria which is just starting differentiation, and they gradually grow.

4. four-month-old embryo.

Under the Bowman's membrane the peripheral distributions are observed in large numbers.

5. five-month-old embryo.

Even in the epithelium the ramus perforans, which have penetrated the Bowman's membrane, are distributed.

6. six to nine-month-old embryo.

The basic structure of the cornea being completed gradually, the corneal nerves develope and increase their ramifications and anastomoses, and the solid nerve-net is formed in the epithelium and the propria.

7. ten-month-old embrro.

In the general growth the cornea is almost equal to that of the adult. The nerves form the various nerve-plexuses, as observed in the adult.

(Published in Acta. O. Jap. 59:1094, 1955 in Japanese)

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STUDIES ON THE PHOSPHATE METABOLISM OF THE CORNEA WITH THE AID OF RADIOAUTOGRAPHY, USING P^{32}

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The history of radioautography was started by Becquerel in 1896, from the fact that the radioisotopes affect the silver bromide crystals of photographic emulsions with their radiation. In 1909, it was used by Mügge to locate, topographically, radioactivity in mineral. About twenty years later, Lacassage, Lattés and Lavedan studied the deposition of a naturally occurring radioelement (Polonium) in the renal tissue of the rabbit. In the last seventeen years, after radioisotopes of elements, which occur normally in tissues, were made artificially, the method has been employed to investigate the localization of radioactive elements in many fields.

In ophthalmology, the radioautography was used primarily by Palm in 1948 (the contact method), to investigate the renewal of phosphate in the rabbit lens. He froze the lenses and cut then into sections of about 200, in thickness, however, it was difficult to cut into sections of uniform thickness. Therefore, the results were insufficient to determine, whether the penetration of phosphate takes place with the same intensity at different parts of the periphery of the lens. Next year, he cut the whole eye ball, here, the contact method was also used. The results showed strong absorption of P^{32} in the anterior uvea (iris, ciliary body and anterior parts of the choroid) and cornea. Sallmann and his co-workers employed the mounting method, using radiosodium. Their radioautographs showed that sodium appeared in the region of the ciliary body, choroid and optic nerve. Potts and his co-workers used the radioautography for the study of the nutritional supply of the cornea, but the photographs were not shown.

It is the purpose of the present work to investigate the localization of phosphate

in the cornea, which is injected into the intraperitoneal space, with the aid of the radioautography, using the stripping and inverting method.

Method

Guinea pigs, weighing about 200 grams, were used. Radiophosphorus, P^{32}, diluted with physiological salt solution, was administered, 0.5 μc per gram of body weight, by intraperitoneal injection. The animals were decapitated nine hours after injection and enucleated. The corneas were excised, washed in physiological salt solution, then held between brain tissues and frozen together. The frozen materials were cut into sections about 40. The corneal sections were spread on water. Slide glasses were slipped under the floating sections to lift them from the water. The preparations, consisting of the sections on the slide glasses, were dried over night, and then coated with celloidin by dipping them twice in a 1 per cent ether-alcohol solution and dried again over night to insure hardening of the celloidin. The preparations were taken to the dark room and the stripping film, FUJI-AUTO-RADIOGRAPHIC PLATE for Stripping Method, then they were thrown into water. In water, the emulsions were slowly peeled off from their glass supports and the preparations were slipped underneath the emulsions, lifted together with the emulsions out of the water. The emulsions were spread in water to a size slightly larger than that of the slide glass, so that the edges may be folded underneath to insure adherence. The slides were then dried by a fan and put in a light-tight box. The latter was stored in a desiccator, which was placed in an ice box during exposure. About two months later, the slides were developed in FUJI RENDOL at 20°C. for 2-4 minutes, with fixation in an acid fixer, FUJI FIX for 5-10 minutes, and then washed for 30 minutes in running water, to prevent buckling and peeling of the emulsions.

Some of the slides were mounted with Canada balsam without staining and studied through a phase contrast microscope. The others were studied through a normal microscope. Here, the inverting method was used, that is, after development, the preparations were placed in water; the emulsions with the tissue sections adhering to their undersurface were lifted from the slides, using a razor blade; the complexes were then inverted and fixed on the clean slide glasses. The free edges of the films were sealed with 1 per cent celloidin and dried completely. The sections were stained with hematoxylin and eosin, while the emulsions were prevented from the action of the dyes, owing to these procedures. The preparations were mounted with Canada balsam.

In these methods, the reduced silver grains of emulsions were closely superimposed on the histologic details, therefore, the location of radioactive elements through a microscope was possible.

Results

Nine hours after the injection, radioactive phosphate was found to be absorbed

almost in the epithelium of the cornea, i.e., the reduced silver grains were closely supperimposed on the epithelium and decreased gradually in number in the substantia propria ; scarcely in the deeper layer of the latter, and also scarcely in the endothelium. (Fig. I, II, III, IV).

The darkening of the silver grains in the central area of the epithelium was less intense than in the peripheral area of it. (Fig. V).

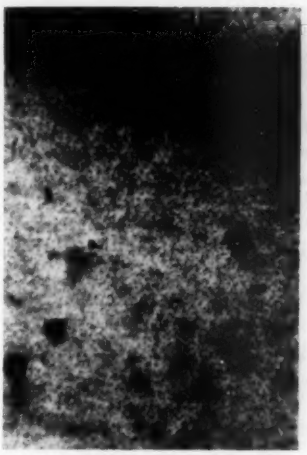

Fig. Ia: A radioautograph of the section of the cornea. (central area)

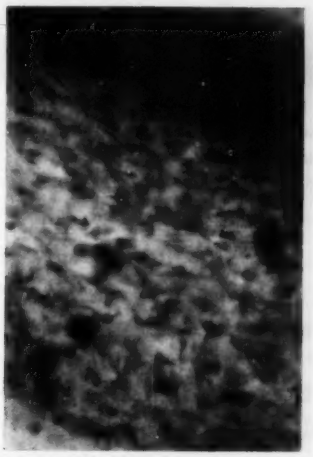

Fig. Ib: A photograph focalized on the corneal section stained with hematoxylin and eosin.

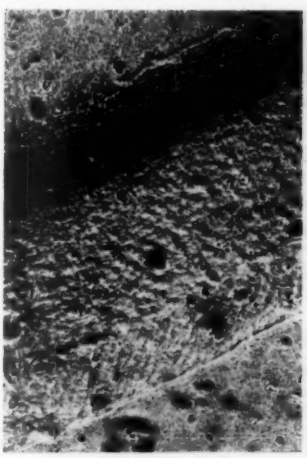

Fig. II: A radioautograph of the section of the central area through a phase contrast microscope without staining.

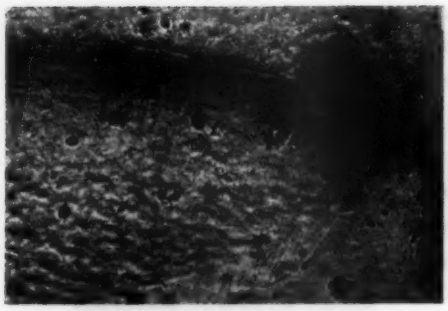

Fig. III: A radioautograph of the section of the peripheral area through a phase contrast microscope without staining.

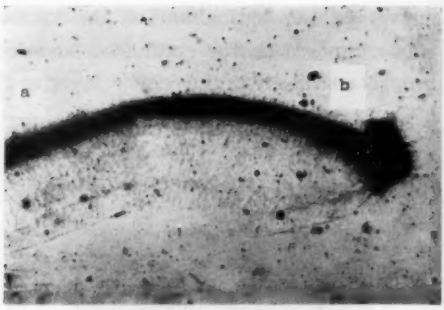

Fig. IV: Comparison of the darkening between the central and peripheral areas without staining.
a: central area
b: peripheral area

Discussion

The origin of the nutritional fluid in the cornea has received considerable attention. It is probable that this fluid enters the cornea from several sources. Gruber, Leber and Laqueur demonstrated this fact. The work of Cogan and Kinsey strengthened the evidence that the cornea receives the greater part of its nutritional fluid from the limbal vascular plexus. Potts and his co-workers obtained the same result. The tears may also contribute to the nutrition of the cornea. (Herrmann and Hickman) It is believed that the cornea, at least under unusual circumstances, receives some nutritional supply from the aqueous.

On the other hand, nine-tenths of the oxygen uptake of the cornea, is consumed in the epithelium and when the respiration of the corneal epithelium alone is computed, it is found, that this is among the tissues with high metabolic rates. It has been shown (Herrmann and Hickman) that the carbohydrate metabolism of the corneal epithelium is similar to that of many other tissues. It consumes glucose, glycogen, lactate and pyruvate. In contrast with the conventional metabolic pattern of the epithelium, the stroma has no oxygen uptake and can utilize neither lactate nor pyruvate. Herrmann and Hickman noticed that the denuded corneal stroma rapidly lost its ability to utilize glucose, and suggested, from an examination of the distribution of phosphate esters in the tissues, that the failure in glucose consumption is due to an exhaustion of the capacity of the tissue to transfer phosphate from glycerophosphate to hexose. It may be concluded, therefore, that the epithelium normally assists the stroma in maintaining a supply of carriers for energy-rich phosphate transfers. The dehydrogenic ferments of the cornea are found 96 per cent in the epithelium, 3 per cent in the endothelium and 1 per cent in the stroma. (Jaeger)

It is reasonable to suppose that phosphate enters into the epithelium in an inorganic form, and transfers to the energy-rich phosphate there, then enters into the stroma

taking part in its glycolysis.

According to the Cogan and Kinsey's theory, the fluid enters into the cornea from the limbal vascular plexus and diffuses at all levels toward the center; eventually, all the fluid will reach the endothelium or the epithelial surface, where the semipermeable character of these membranes acts as an efficient barrier to electrolytes within the cornea. Davson has based his objections to this theory on the facts elicited in experiments to determine the salt content of the ox corneas. He believes that an interchange of sodium, potassium and chloride between aqueous and substantia propria is possible by osmosis.

Although the present work is done merely in a period of nine hours after the intraperitoneal injection, the radioautographs obtained may show, that there exists neither the diffusion nor osmosis the above authors have mentioned. If the phosphate diffusion should be present in the cornea as in the Cogan and Kinsey's theory, the reduced silver grains of the emulsions would be found more diffusely in all layers. While, at the period of nine hours, phosphate entered so much into the epithelium, but scarcely in the stroma and endothelium. That is, phosphate does not enter into the stroma from the limbal vessels, but does into the epithelium; and it does not diffuse into other layers, but does toward the center within the epithelium, against the Cogan and Kinsey's theory. Similarly, if the corneal stroma should be in an osmotic equilibrium with the aqueous, the deeper layers of the stroma would contain much more phosphate. But, in fact, there were scarcely. On the other hand, it was found, in an other work, that at the period of nine hours, the radiophosphate content of the aqueous was much larger than in the cornea (in rabbit eyes). Therefore, it is reasonable to conclude that phosphate does not diffuse into the cornea from the aqueous, so far the accuracy of the experiments are concerned.

In brief, the cornea is supplied with phosphate from the limbal vascular plexus; phosphate enters into the epithelium in an inorganic form, and is transfered to the energy-rich phosphate there; these are neither simple diffusion nor osmosis.

Summary

1. Radioautographic techniques (the stripping and inverting method) were used to study the phosphate metabolism of the cornea of the guinea pig.

2. At the period of nine hours after intraperitoneal injection, the reduced silver grains of the emulsions were closely superimposed on the epithelium, and decreased gradually in number in the substantia propria, then scarcely in the endothelium. That is, radioactive phosphate entered almost into the epithelium.

3. The darkening in the peripheral area of the epithelium was more intense than in the central.

4. It may be concluded that phosphate enters into the epithelium from the limbal

vascular plexus, and is consumed almost there.

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STUDIES ON THE GANGLION CELLS IN THE ROOTS OF THE IIIrd., IVth. AND THE VIth. CRANIAL NERVES. REPORT I

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There has been up to the present day much confusion over the nature of the proprioceptive supply of the extrinsic eye muscles and even over the existence of such an afferent system in these muscles. In reading the literature upon the cells of origin of the first neurone from an eye muscle receptor, it is readily apparent that they may lie in the scattered cells and small ganglia known to lie along the third and sixth nerves, or inside the brain-stem (in the eye muscle nuclei or the mesencephalic nucleus of the fifth nerve).

The ganglion cells had been found in the roots of the IIIrd, IVth. and VIth. cranial nerves of man, monkey, dog and teleost by Thomsen (1887), Tozer (1912), Nicholls (1915), Nicholson (1924) and Clark (1927), however, the nature of these cells had not been fully confirmed by these investigators.

It is the purpose of the present work to study on the distribution and the nature of these ganglion cells in these nerves of the rabbit.

Material and Method

The materials are the roots of the IIIrd, IVth. and VIth. cranial nerves between their exit from the brain and their inlet into the muscles. These nerves had been removed from freshly killed adult rabbits and fixed about one month in a solution of 10 per cent neutral formalin or 96 per cent alcohol. All materials were embedded in paraffin and cut in serial sections. For staining, silver impregnation (by Bielschowsky) was used in seven cases which were fixed in formalin, staining method of Nissl substance

(By Thionin) in five ones, which were fixed in alcohol, hematoxylin and eosin in one, which was fixed in formalin respectively.

Observations

In the root of the VIth. nerve, about 36 ganglion cells were counted, in the IIIrd. nerve of which the ciliary ganglion was removed, about 43 and in the IVth. nerve, some 100 cells. These cells were found in all cases of the IIIrd., VIth. nerve, and in only one case of the IVth. nerve. The cells were always found inside the orbit along the roots of these nerves, and most of them were located at the position where the muscle branches bifurcate from the main trunk.

The size of the majority of these cells varied from 25 to 32 micron in diameter. By Bielschowsky's method, the neurits of these cells were seen to extend along the root of the nerve, after forming a glomerule around the body of cells, and yet, a T type bifurcation could not be found which seemed to be the neurite of the sensory cells. Judging from the type of Nissl-granules, some of them seemed to be rather of the sensory type than of the autonomic, but the other cells had a resemblance to the ganglion cells of the ciliary ganglion. According to the arrangement of Nissl-granules, these cells are classified in three types, but all cells may be having an equal function. The types are as follows:

Type *S*: The cells are large, clear, finely granulated. They resemble the spinal ganglion cells, and can be found in about half as many as these cells.

Type *V*: The cells are smaller in size and possess larger granules than the former. Another half of all cells are of this type, which may be of the autonomic kind.

Type *C*: The cells are small, having very diffuse, fine granules and over stained cytoplasm. They are encountered very rarely and their function is not clear.

In addition, the ciliary ganglion was found to be composed of two parts which were *A* and *B* of the IIIrd. nerve in Fig. I and to contain from 101 to 388 ganglion cells, the majority of which are multipolar cells.

Discussion

The results of this experiment show conclusively that the ganglion cells are found also on the roots of the IIIrd. IVth. and VIth. cranial nerves of the rabbit as well as other animals which were described in the works by previous authors.

In 1887 R. Thomsen noted the presence of several ganglion cells in the roots of the IIIrd., VIth. cranial nerves of a human child, but he could not find them in the IVth. nerve. By Tozer the cells were counted from 0 to 29 in the IIIrd. between 9 and 34 in the VIth. nerve of macacus rhesus and 80 cells in the IIIrd., 30 in the IVth. and from 32 to 39 in the VIth. root of teleost (Gadus virens).

Nicholls reported that 15 ganglion cells were found on a root of the IIIrd. nerve in Scyllium canicula, and Nicholson noted the presence of 30 cells on the root of the VIth.

and 14 cells on the IIIrd. in a human foetus. Clark stated that a number of cells were found along each IIIrd. cranial nerve, but none were found on either the VIth. or the IVth. nerves.

As mentioned above, there is a great variation in number of these cells, and Tozer stated that this fact renders an explanation of their nature and function impossible. These ganglion cells were found in the connective tissue which formed the margin of the nerve trunk within the orbit, and were aggregated at the places where the muscle branches bifurcate. Nicholson reported that these cells were found within the orbit, Tozer stated that they were seen in the root of nerves between their exit from the brain and their passage through the dura, but other workers had not noted the position of these cells.

As to the nature of these ganglion cells, Clark stated that these cells were small or medium in size, possessed medium-sized granules scattered evenly through the cytoplasm or a peripheral ring of granules, and resembled the type described for the ciliary ganglion more than the type related to muscle spindles in his reports. However, in the present work they were medium in size, and seemed to contain the sensory and the autonomic type of cells, judging from the distribution of Nissl-granules. Of course, it is impossible that these cells can be distinguished between afferent and efferent by only size and distribution of Nissl-granules. These ganglion cells were loosely laid on the main trunk of the oculomotor nerve and sent out their neurits into the roots of nerves, after raveling as a glomerule around the body of cells.

The function of these cells has not been completely clarified at present. Thomsen assumed, that these positions of the ganglion cells were an embryological anomaly, but Nicholson, on the other hand, strongly claimed that these cells were sensory. Tozer stated that the number of the cells in Gadus were possibly the source of the afferent fibers, but in Macacus the nature of these cells seemed to be impossible to determine owing to their rarity. Clark related that even if the cells under discussion are concerned with muscle sense, the question still remains unsettled as to the location of the majority of the proprioceptive fibers to the eye muscles, at least in those cases in which few or no cells were found along the nerves.

Apart from some above mentioned difficulties, it is obvious that there exist some ganglion cells along the IIIrd., IVth. and VIth. cranial nerve of the rabbit, and they must have their function. If these cells are not the proprioceptive ones, these cells will be the autonomic as well as the cells of the ciliary ganglion. It is clarified in this report, that the ciliary ganglion of the rabbit is composed of two parts.

In this report the nature of these ganglion cells is not yet ascertained. This paper is a preliminary report of investigation which will necessarily take many other experiments before final conclusions are reached.

Summary

1. Some numbers of ganglion cells in the root of the rabbit's ocular motor nerves

are, demonstrated by means of serial sections stained by the silver impregnation and Nissl's staining methods.

2. In all cases of the IIIrd., and VIth. nerve, about 40 ganglion cells were counted, and in only one cases of the IVth. nerve about 100 cells.

3. The cells were always found inside the orbit along the roots of these nerves, and most of them were located at the position where the muscle branches bifurcate from the main trunk.

4. The size of the majority of these cells varied from 25 to 32 micron in diameter. The neurits of these cells were seen to extend along the root of the nerve, after form in a glomerule around the body of cells, but it could not be made sure that these cells were unipolar.

5. Judgng from the arrangement of Nissl-granules, about one half of them seemed to be of the sensory type, and the other half resembled to the autonomic cells.

6. The ciliary ganglion was found to be composed of two parts, and to contain about 250 multipolar ganglion cells.

(Published in Acta. O. Jap. 59:958, 1955 in Japanese)

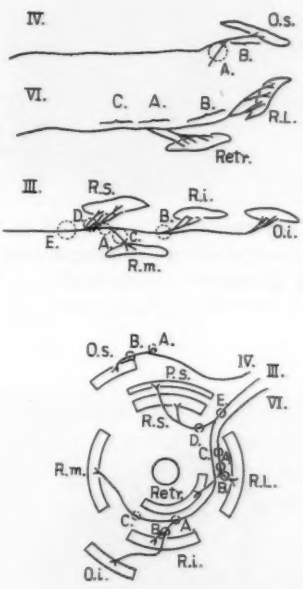

Fig. 1 Diagram showing the position of the ganglion cells. O.s.; M. obliquus superior O.i.; M. obliquus inferior P.s.; M. palpebralis sup. R.m.; M. rectus medialis R.s.; M. rect. sup. R.l.; M. rect. lateralis R.i.; M. rect. inf. Retr.; M. retractor bulbi

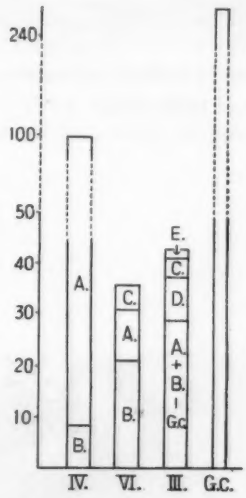

Fig. 2 Mean value of the number of ganglion cells at each part of their position.

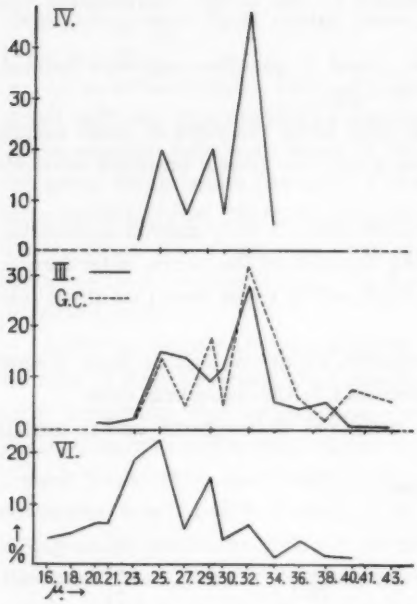

Fig. 3 Relation between the number and diameter of these cells.

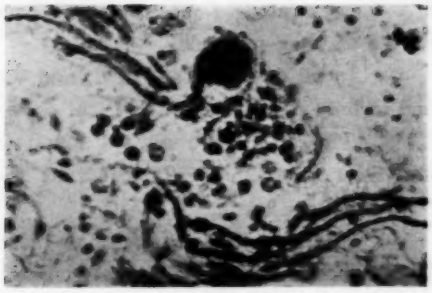

Fig. 4 A ganglion cell beside many medullated nerve fibers within the root of the VIth, cranial nerve. (Bielschowsky's silver method)

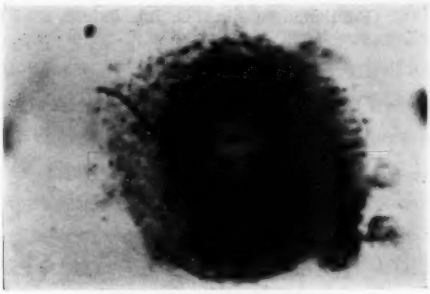

Fig. 5 A ganglion cell in the root of the VIth. cranial nerve. (Bielschowsky's silver method)

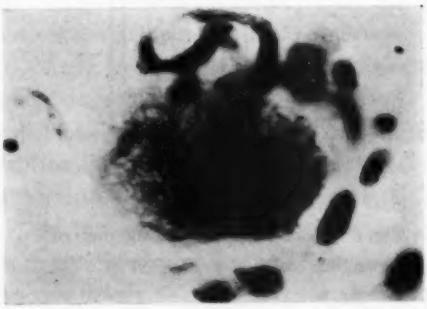

Fig. 6 A photograph showing the neurits, forming a glomerule around the body of cells. (Bielschowsky's silver method)

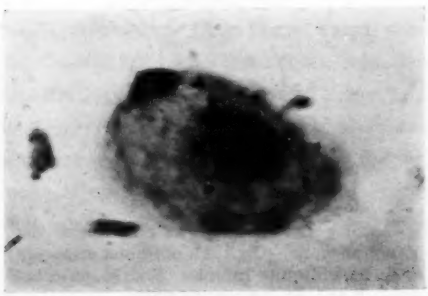

Fig. 7 The cells of type S. found in the IIIrd. nerve. (Nissl's staining)

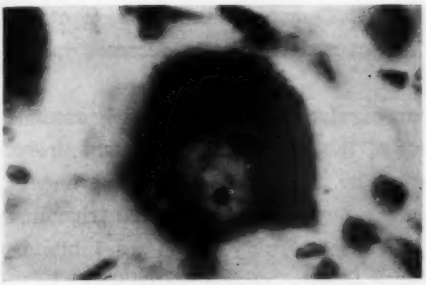

Fig. 8 Cells of type type V. found in the VIth. nerve. (Nissl's staining)

Fig. 9 Cells of type C. found in the IVth. nerve. (Nissl's staining)

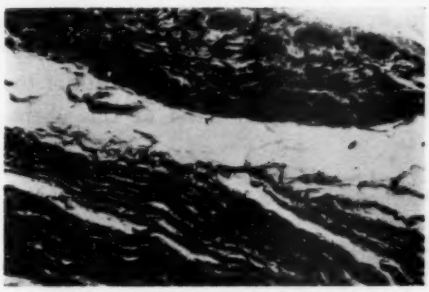

Fig. 10 The ciliary ganglion found on part A of the IIIrd. nerve. (Bielschowsky's silver method)

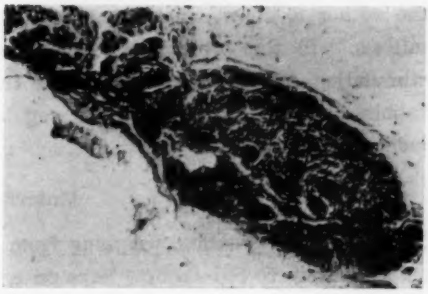

Fig. 11 The ciliary ganglion found on part B of the IIIrd. nerve. (Bielschowsky's silver method)

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THE EFFECTS OF THE DENERVATION AND THE STIMULATION OF THE SYMPATHETIC AND THE TRIGEMINAL NERVE ON THE MITOTIC RATE OF THE CORNEAL EPITHELIUM IN THE RABBIT

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Introduction

Although many anatomical studies strongly support the existence of the sympathetic innervation of the cornea, little has been done on its physiological aspects, except the

elaborate work of J.S. Friedenwald and W. Buschke[4]. They found that the sympathetic denervation and the administration of adrenaline caused a mitotic decrease in rats corneal epithelium.

On the other hand, the occurrence of the neuroparalytic keratitis, formerly assumed to be due to the deprival of the trophic influence of the trigeminal nerve, was proved not to be an immediate sequence of the denervation by the preliminary experiment[6]. In the cornea, no analytical studies bearing on the direct influence of the trigeminal nerve had been done, until S. Sigelman and J.S. Friedenwald[3] found an inhibitory effect of the sensory denervation on the mitotic rate of rats corneal epithelium.

In spite of these elaborate works, the present knowledge on the physiological aspects of the corneal innervation is still limited. It was, therefore, of particular interest for the author to know, whether nervous stimulation exerts some influence on the corneal mitosis. In the present work, the conspicuous effects of the denervation and the stimulation of the sympathetic and the trigeminal nerve on the mitotic rate of the rabbits corneal epithelium were recognized and the physiological basis of this phenomenon was discussed.

Materials and Methods

White adult rabbits, weighing from 1.5 up to 2.3 Kgm. in body weight, were used, after they were fed about 7 days on a definite diet. The denervation and the stimulation of the nerve were carried out on the left side under local anesthesia; the right side served as a control. The effects of the operation were examined by comparison of mitotic activities of both corneas of the same animals, because it was ascertained by the preliminary experiment[5] that the difference of mitotic activities of both corneas of the same animal was statistically insignificant in normal conditions. To avoid unexpected errors possibly induced by diurnal variation of the mitotic activity, animals were sacrificed from 11 to 13 o'clock in the experiments of longer duration, by intracordial injection of concentrated alcohol. The experiments of shorter duration were carried out from 9 to 17 o'clock.

Both eyeballs were enucleated immediately after the death of animals and fixed in McClintock's solution. The flat preparations of the cornea were made by cutting eight peripheral incisions. They were stained by orcein acetic acid solution, rinsed by a solution of glacial acetic acid and mounted in xylol balsam after the routine dehydration. All stages of the mitotic cycle were distinctly visible in the basal layer of the epithelium notwithstanding the thickness of the flat preparations having whole layers of the cornea.

The method of assaying mitotic activity consisted in counting mitotic figures in the area of 3×3 mm^2 in the center of the flat preparations, the errors being 17.5%.

The mitotic rate was assayed by counting mitoses in the standard area four hours after the intramuscular administration of 7 mg. per Kgm. of body weight of colchicine.

The mitotic rate represents the rate of entrance of cells into mitosis, because it was ascertained by the preliminary experiment[7] that colchicine did not, in such dose, interfere with the initiation of mitosis, while its progress was entirely interrupted.

Experimentals

The effects of the sympathetic denervation. The mitotic rates were assayed at intervals of 6,12,16,20 hours, 2 days, 2 weeks and on to 3-4 weeks after the removal of the superior cervical sympathetic ganglion. The results are illustrated in Fig. 1. As shown in the figure, the mitotic rate increases slightly after the operation, then decreases abruptly after a lag poriod of about 16 hours. These results seem to be in accordance with that of J.S. Friedenwald and W. Buschke. The physiolgical basis of the occurrence of mitotic decrease will be discussed later.

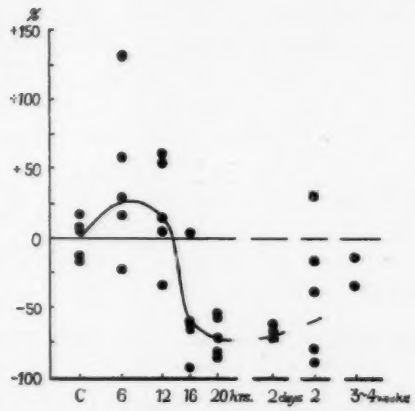

Fig. 1: Mitotic rates after the sympathetic denervation: Ordinate; Mitotic increase and decrease in per cent, Abscissa; Intervals after the denervation, C; Normal control, Line; Time course of average: A conspicuous decrease of the mitotic rate takes place after a lag period of 16 hours, representing an appearance of supersensitivity.

The effects of the sympathetic stimulation, carried out with fixation of animals. The superior cervical sympathetic ganglion was stimulated using square wave pulses of 1.5 volts, 10 ms. in duration and 20 cps. in frequency. The electrode, devised specially for this purpose, is illustrated in Fig 2. The left cervical portion of animals, fastened to a holder, was incised and the sympathetic ganglion was isolated from the surrounding tissue, great care being taken not to injure the principal anastomoses and the vessels. The electrode was inserted from medioventral side behind the ganglion and immobilized by application of adequate pressure. The ganglion frequently revealed patchy hemorrhages especially in the lower region at the time of post mortem exam-

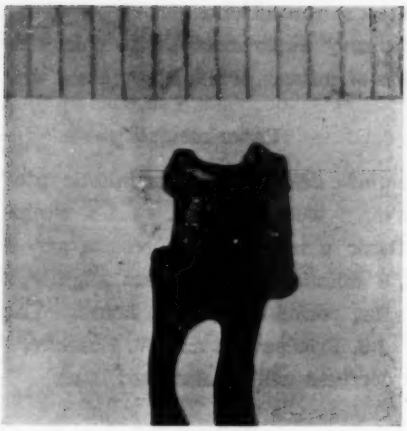

Fig. 2: The implanting electrode, made of vinyl-tube and silver wire. Scale; 1 mm.

Table 1
The sympathetic stimulation with fixation of animals

Animals' Number	mitosis counts					
	1	2	3	4	5	Total
Controls	9	125	17	27	23	201
Stimulated side	3	2	3	0	1	9

ination, but the stimulation with this electrode was highly effective on the pupil and the conjunctival vessels.

The electrical stimuli, each 1.5 minutes in duration, were repeated 2 times with an interval of 10 minutes, then colchicine was injected. The animals were sacrificed 4 hours thereafter for mitosis counts; during this time, 14 stimuli were repeated with an interval of 15 minutes. The results are given in table 1. This indicates that abnormally low mitotic rates are common in this series of experiments. However, mitotic rates on the operated side were invariably much lower than that of the control side.

These results may be accounted for by adrenal response in restricted conditions. As found by J.S. Friedenwald and W. Buschke[4], the administration of adrenaline and the various excitements inhibit the corneal mitosis. On the other hand, W.B. Cannon and A. Rosenblueth[3] laid a particular emphasis on the generalized reaction of the sympathetic nerve based on the experimental fact that local and circulating sympathin and adrenaline cooperated in conditions of extreme cold, hunger and restriction etc.. These facts support the interpretation of the occurrence of abnormally low mitotic rate just described above.

The effects of the sympathetic stimulation, carried out without fixation of animals. The occurrence of the generalized reaction of the sympathetic nervous system, mentioned above, suggests that it is difficult to discuss the effects of the sympathetic stimulation unequivocally on the basis of the experiments performed with the fixation of animals. The animals should, therefore, be free from pain and various influences of circumstance during the experiment. The practicability of such an experiment depends first on the presence of pain conducting afferent neurones in the cervical portion of the sympathetic nerve. W.B. Cannon[2] and others reported the absence of pain conducting neurones in the cervical sympathetic nerve. However, H.G. Bishop and P. Heinbecker[1] believe that afferent nerve fibers run in the cervical trunk and are distributed in the heart. The present experiment, in which animals exhibited no signs of distress by stimulation, support the view of W.B. Cannon and others.

The electrical stimuli, 15 seconds in duration, were applied repeatedly to the superior cervical sympathetic ganglion, without fixation of animals, by the use of the electrode, which had been implanted previously for one hour with interruption of preganglionic fibers; and corneal mitoses were counted under the use of colchicine, as described above.

The results are shown in table 2 and in Fig. 3. In these series of experiments, mitotic rates remained almost at normal level. The inhibitory effect of the sympathetic stimulation is clearly demonstrated in the figure.

Table 2

The sympathetic stimulation without fixation of animals

Animals' Number		mitosis counts						
		1	2	3	4	5	6	Total
Stimulation of sup. cerv. symp. ganglion	Controls	142	116	152	57	251	473	1191
	Stimulated	58	51	57	45	110	368	689
Controls, preganglionic fibers being interrupted	Controls	151	387	692	713	672	—	2615
	Operated	156	377	608	573	382	—	2096

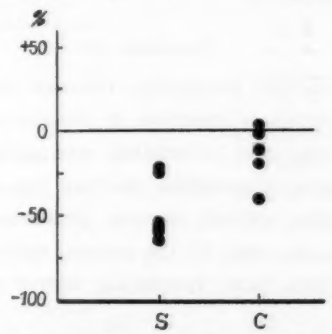

Fig. 3: Mitotic rates after(S)the electrical stimulation of the superior cervical sympathetic ganglion, without fixation of animals, C; Controls, in which the preganglionic fibers were amputated, Ordinate; Mitotic increase and decrease in per cent

The effects of the trigeminal denervation. The trigeminal (ophthalmic) nerve was amputated in its intracranial course, by trephining of the skull in the parieto-temporal region; blepharorraphy was done on the operated side to avoid corneal injuries. Mitotic activities and rates were assayed at intervals of 4,6,20 hours, 2,3 and 4 days after the operation. The results are illustrated in figs. 4 and 6. As shown in the figure, the mitotic activity of the denervated side decreased slightly. But the mitotic rate decreased more markedly and abruptly after a lag period of about 4 hours. Higher mitotic activities were frequently encountered in cases in which the epithelial wounds were found. This suggests that the mitotic proliferation takes place in wound-healing independently of the trigeminal denervation. S. Sigelman and J.S. Friedenwald studied the influence of sensory denervation on the corneal mitosis and obtained the results similar to that of the present experiment.

The effects of the electrical stimulation of the trigeminal nerve. The stimulation of the trigeminal (ophthalmic) nerve was carried out by the use of a needle electrode inserted through a small hole placed on the parietal bone of the fixed animals.

The electrical stimuli, each 1.5 minutes in duration, using square wave pulses of 0.9 volt, 4.2 ms. and of frequencies varying from 5 up to 70 cps., were applied repeatedly under the use of colchicine as described previously, and the animals were sacrificed for mitosis counts. The results are shown in figs. 5 and 7. The mitotic rates on the stimulated side were higher when stimulated with pulses of 10 and 5 cps. However, the mitoses found in the definite area were less abundant as compared with the control (normal case). This was probably due to a reflectional response to painful stimuli. The results of this experiment are somewhat complicated, but it may be well accounted for, by assuming an antagonistic process to the reflectional decrease of mitoses on the stimulated side. On the other hand, in the cases which were stimulated with pulses of 50 and 70 cps., abnormally low mitotic rates were frequently encountered both on the stimulated and on the control side; in this case, it was difficult to compare both sides with each other with certainty.

Discussion

In discussing the effects of local sympathin, released by nervous stimulation, one should be aware of the generalized reaction of the sympathetic nervous system, i.e. the spatial summation of local and circulating sympathin and adrenaline. In the present study, the sympathetic stimulation, carried out without disturbance of the behavior of animals and also without serious generalized reaction, resulted in a marked decrease in the mitotic rate of the corneal epithelium. It seems, therefore, to be reasonable to assume that local sympathin exerts an inhibitory effect on the cornal mitosis.

The occurrence of the mitotic decrease following the sympathetic denervation is,

(A part of maxillary nerve was also interrupted simultaneously.)

therefore, considered to be related to the supersensitivity of the denervated tissue to sympathomimetic substances. This assumption may also be supported by the fact that the lag period of the mitotic decrease is in good accordance with that of the appearance of supersensitivity in other tissues innervated by the sympathetic nerve.

The trigeminal denervation causes also a mitotic decrease, but the time-lag is much shorter than that of the sympathetic denervation. The changes of the mitotic rate caused by the trigeminal stimulation are related to several factors; generalized reaction of the sympathetic nervous system to painful stimuli and restriction may supervene to the immediate effect of the stimulation. These datas are, however, not far from the assumption that some unknown factors, presumably antagonistic to the sympathetic effect, may play a role, when the trigeminal nerve is stimulated. In order to know the immediate effects of the nervous stimulation, experiments should be performed without serious reflectional reaction. In the trigeminal nerve, the difficulties in doing such an experiment prevent the problem from being entirely solved.

These experimental results, however, suggest that the nervous system plays an important role in the proliferation of tissues.

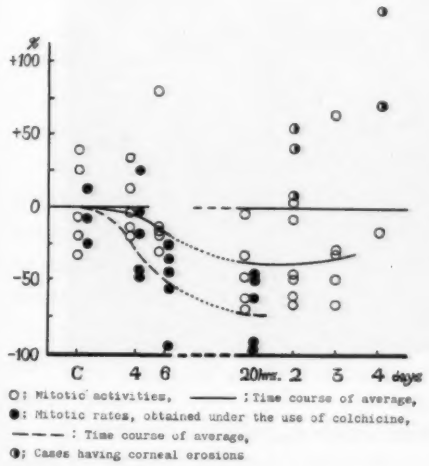

Fig. 4: Changes in mitosis after the trigeminal amputation: Ordinate; Mitotic increase and decrease in per cent, Abscissa; Intervals after the denervation, C; Controls in which blepharorraphy was done for 20 hours

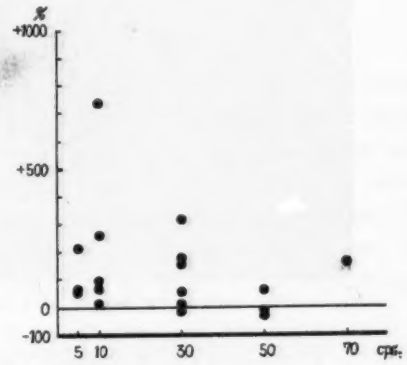

Fig. 5: Changes in mitotic rate following the trigeminal stimulation; Ordinate; Mitotic increase and decrease in per cent, Abscissa; Frequencies of electric pulses in cycle per second

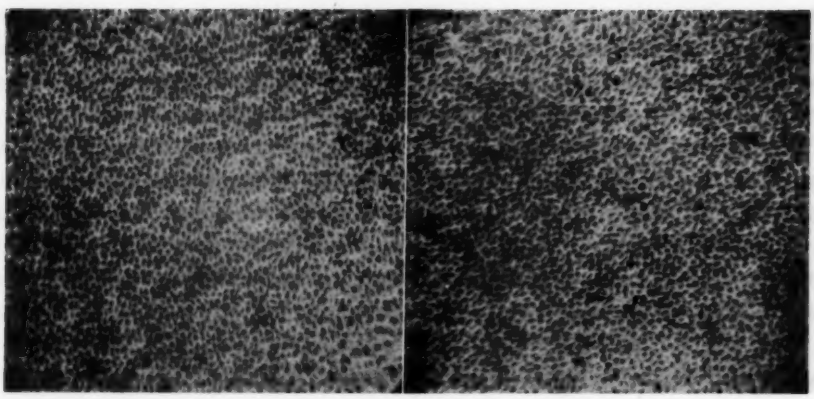

Fig. 6: The corneal epithelium of a case deprived of the trigeminal nerve supply for 20 hours (under the use of colchicine).
A; Operated side, B; Control Arrows indicate mitotic figures.

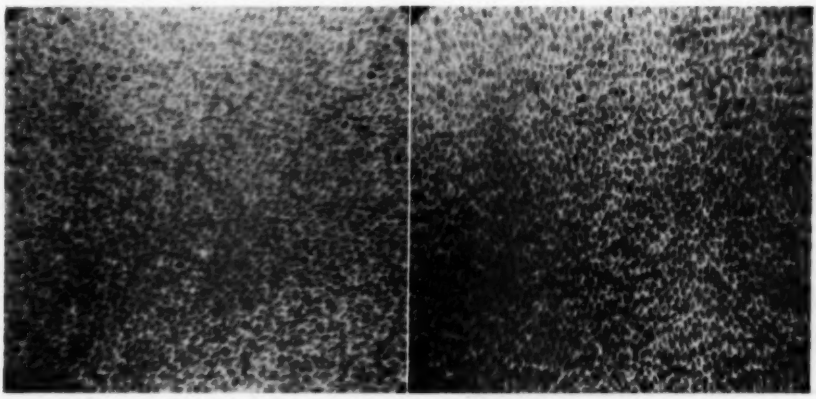

Fig. 7: The corneal epithelium of a case, the trigeminal nerve being stimulated with pulses of 5 cps. (under the use of colchicine).
A; Operated side, B; Control Arrows indicate mitotic figures.

Summary

1. The effects of the denervation and the stimulation of the sympathetic and the trigeminal nerve on the mitotic activity of the corneal epithelium have been studied in rabbits.

2. The sympathetic denervation causes slight mitotic increase, which is followed by a marked decrease after a time-lag of about 16 hours.

3. The sympathetic stimulation causes a marked decrease in the mitotic rate.

4. The trigeminal denervation results in a mitotic decrease, starting after a time-lag of about 4 hours.

5. The mitotic rate remains at a higher level than that of the control, when the trigeminal nerve is stimulated with pulses of low frequencies.

6. The effects of sympathin and the trigeminal nerve on corneal mitosis, probably antagonistic to the sympathetic nerve, have been discussed.

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PATHOGENESIS OF ANOMALOUS CORRESPONDENCE

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For many years discussion has proceeded upon the development of anomalous retinal correspondence and there has been two opposing theories; the *Adaptive theory* which maintained that anomalous correspondence was almost certainly a reaction on the part of the organism to an abnormal position of the eyes, and the *Nativistic theory* which claimed that anomalous correspondence was a congenital sensory anomaly and might be the cause of squint. The general consensus of opinion would appear to support the the former and the treatments have been performed on the basis of this view, though there remained some doubt.

In previous reported stuies, anomalous correspondences were relatively rare in divergent squint and therefore observations were made chiefly upon the cases of the convergent squint which had good reason to regard anomalous correspondence as an adaptative phenomenon. In Japan, however, anomalous correspondences are found most commonly in the divergent squint and rather rarely in the convergent squint. It is the purpose of this study to investigate the pathogenesis of anomalous correspondence especially in the patients of divergent squint.

Materials and Methods

Six hundred cases of concomitant squint form the basis of this study. All patients

were examined by the double-image test, after-image test, and with the haploscope before and after treatment.

Observations and Comments

Table 1 shows the percentage of each type of correspondence found in the cases taken as a whole.

According to previous papers anomalous correspondence has been found particularly frequently in the cases of the convergent squint because anomalous correspondence develops usually in the squints which begin at an very early age and continue for some duration with the constant angle of squint. In table 1, however, it is shown that the vast majority of the patients with anomalous correspondence are found to be divergent and only 20% of them to be convergent; the fact is inconsistent with the previous findings.

Intermittency. In a series of 194 cases of exotropia with anomalous correspondence 81 cases (73%) were found to be intermittent and only 37 cases (27%) to be constant (table 2).

Table 1. Percentage incidence of correspondence in a series of 600 cases

correspondence	convergent		divergent		total	
	number	per cent	number	per cent	number	per cent
normal	227	83	134	41	361	60
anomalous	45	17	194	59	239	40
total	272	100	328	100	600	100

Table 2. Relation of correspondence to intermittency in the divergent squint

	correspondence			
	normal		anomalous	
	number	per cent	number	per cent
intermittent	35	26	157	81
constant	99	74	37	19
total	134	100	194	100

Some authors have described the cases of intermittent exotropia which have a good grade 3 fusion, when the eyes are parallel and exhibit anomalous correspondence when the eyes are manifestly divergent due to divergence excess. Such intermittent exotropia with both normal and anomalous correspondence was found in 152 cases or 25% of total 600 Patients.

In convergent squint periodicity is relatively common amongst the normal correspondence cases and the anomalous correspondence cases very seldom have a periodic

squint; therefore it has been believed that anomalous correspondence can scarcely develop in an intermittent squint and the fact has supported the adaptive theory.

In divergent squint, on the contrary, intermittency is exceedingly common in the anomalous correspondence group and rather rare in the normal. In other words, it seems to be, that anomalous correspondence cases may be intermittent as a rule and the adaptive theory is not adequate in explanation of the fact.

Age of onset. It has been believed that anomalous correspondence have been found most commonly amongst those patients who had commenced to squint in very early years. While in the divergent squint with anomalous correspondence 60 cases or 31% of them had delayed onset after the 6th year (table 3).

Table 3. Age of incidence in the cases of a divergent squint with anomalous correspondence

age of onset	number	per cent
since birth	22	11
0～ 6 years	87	45
6～12 years	40	21
12 years～	20	10
unknown	25	13
total	194	100

It is frequently difficult to determine with accuracy at what age a squint began and so it seems to be doubtful in above findings, but about the existence of such anomalous correspondence cases with later onset, Duke-Elder has already pointed out in his textbook. Now in following cases the age of onset was determined accurately from their histories.

Case 1. Miss K., age 25, was operated on when 10 years of age and then became divergent intermittently. Now she has an anomalous correspondence in the divergent position.

Case 2. A girl T., age 14, commenced her convergent squint suddenly when 3 years old. A tenotomy of the left internal rectus was performed at the age of 9. After surgery intermittent sursumdivergent squint occured and now she has an anomalous correspondence in the sursumdivergent position.

Case 3. Miss S., age 18. She, her elder brother and younger sister all had had convergent squints since birth. She was operated on at the age of 8 and then her eyes became divergent gradually. Her brother was operated on when he was 11 years old and since then his eyes have been straight. Her sister has never been operated on. Now her brother and sister still have anomalous correspondences in the convergent position, but the patient has a divergent position by contraries.

In above cases age of onset of the divergent squint is strictly determined and it seems too late to develop an anomalous correspondence as an adaptation to the divergent position of the eyes. While some authors have described almost the same cases in which anomalous correspondence was found in cases of convergent squint and how the cases were operated upon and became divergent, the correspondence eventually becoming again abnormal in the divergent position; they have suggested that anomalous correspondence may change after operation. It seems to be unreasonable, however, that a new type of anomalous correspondence can develop after the 6th year.

Amblyopia and anomalous correspondence. In the series of 239 cases with anomalous correspondence amblyopia was found in 15 cases (6%). It was interesting, that some of the patients with amblyopia were troubled by paradoxic diplopia when the eyes became straight by operation.

According to the adaptive theory, anomalous correspondence and amblyopia must be mutually exclusive and above findings suggest that further consideration upon the matter is yet needed.

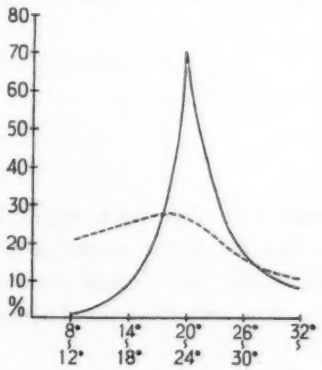

Fig. 1. Relation of the angle of squint to the correspondence in a divergent squint

—— anomalous correspondence cases

..... normal correspondence cases

Angle of the squint. Figure 1 shows a striking relationship existing between the type of correspondence and the angle of squint in a divergent squint. In 70% of the cases of divergent squint with anomalous correspondence the angle of squint was found to be at 20 to 24 degrees. Even in a case with the angle of squint of 12 degrees, the angle of anomaly was found to be at 20 degrees.

The fact suggests, that the anomalous correspondence group is not derived from the normal correspondence group and that the angle of squint in anomalous correspondence cases may be regarded as an index of the degree of a certain divergence innervation which seemed to be an anomalous correspondence itself.

Refractive curve. Figure 2 shows the refractive curve in a divergent squint. The curve of the cases with anomalous correspondence had no specific significance as compared with the curve of nonsquinting people, while in the group with normal correspondence, myopes were found fairly more. In a convergent squint almost the same differences between two groups were found but in normal correspondence cases hypermetropes were found more.

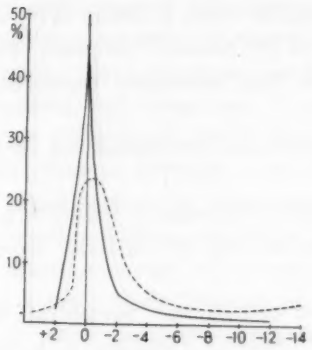

Fig. 2. Distribution of cases of divergent squint according to refraction
—— anomalous correspondence cases
····· normal correspondence cases

These findings suggest, that the anomalous correspondence cases are essentially different from those cases with normal correspondence and each group belongs to the different category in origin.

Cause of the squint. In a convergent squint it is relatively difficult to determine the cause of the squint, while in a divergent squint the factors which have influences on the occurence of ocular deviation are more simple.

In Table 4, the cases of the divergent squint have been grouped according to the cause of squint. In the group with normal correspondence most patients had some obvious cause for squinting, whereas in the majority of the cases with anomalous correspondence the cause of squint could not be determined.

Table 4. Distribution of cases of the divergent squint according to cause

cause of squint	normal correspond.		anomalous correspond.	
	number	per cent	number	per cent
obvious	105	78	49	25
undetermined	29	22	145	75
total	134	100	194	100

The close tie-up between a squint of an undetermined cause and the presence of anomalous correspondence raises the question, as to which is the cause, and which is the effect. According to some authors, such undetermined cause may be a so called divergence excess; if this is true, one can say that anomalous correspondence develops predominantly in the cases of divergence excess. On the other hand, it is possible to consider, that the cause of a squint of undetermined origin is anomalous correspondence itself and that the latter is the cause of abnormal divergence innervation such as divergence excess. Correlation between divergence excess and anomalous correspondence will be discussed later.

Heredity of anomalous correspondence. It has been believed that the hereditary tendency of anomalous correspondence could not be observed and there are few statistics dealing adequately with the matter. Whereas in 15 cases of a divergent squint, anomalous correspondence appeared to have hereditary influence. Figure 3 shows the family trees

of the divergent squint with anomalous correspondence, in which a history of the squint was found in parent, brother or sister, uncle or aunt of the patient. Although the type of transmission could not be clarified, it is noticeable that anomalous correspondence seemed to be congenital in origin.

In a convergent squint, however, heredity of anomalous correspondence could not be demonstrated.

If anomalous correspondence was a congenital sensory anomaly, it should be a more primitive type of sensory relation and this assumption will be inferred from the following experiment.

Panoramic vision. Figure 4 shows the acuity of central and peripheral distant vision of the deviating eye which is tested under following condition in a case of divergent squint of 20 degrees with anomalous correspondence. The patient who retains

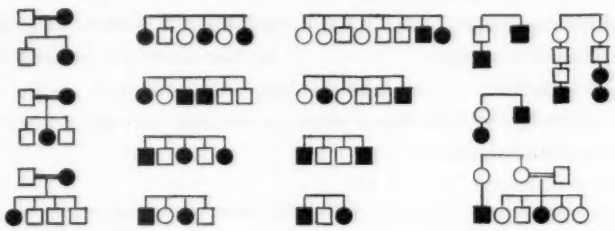

Fig. 3. Heredity of anomalous correspondence in a divergent squint
■ affected male
● affected female

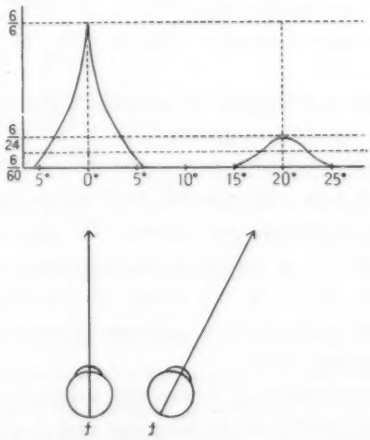

Fig. 4. Panoramic vision in an anomalous correspondence case. Abscissae; degrees towards the periphery of the visual field. Ordinates; visual acuity tested when the left eye is fixing the test letter of 6/6 at 0°.

normal good vision in both eyes is reading 6/6 with his fixing left eye and at the same time another test letter is placed at the position towards which the squinting right eye is directed. Then the patient is asked whether he can read the letter without moving or changing the fixing eye. Figure 5 shows that 6/24 is read by the patient with his right eye and that his two foveas are turned toward different loci of space which usually contain different patterns. The findings suggest that anomalous correspondence is a more primitive form of visual organization such as panoramic vision.

Correlation between divergence excess and anomalous correspondence. In the cases of intermittent exotropia with both normal and anomalous correspondence the patient who is fixing a small light has no diplopia when the eyes are parallel, but if a coloured filter is placed in front of one eye this patient would have uncrossed diplopia (figure 5. a), and then according as the divergence movement of the eye the coloured image moves toward the fixing light (figure 5. b). If the adnormal innervation to diverge the eyes is due to divergence excess only, the patient should have crossed diplopia. Above phenomenon, however, shows that anomalous correspondence may be present before the divergent squint is manifest and may enforce the divergence movement; therefore the abnormal divergence innervation so called divergence excess seemed to be anomalous correspondence itself.

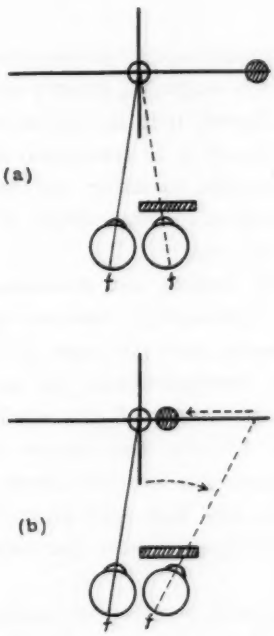

Fig. 5. Diplopia in a case of intermittent exotropia with anomalous correspondence. (a); Coloured filter is placed in front of the right eye, when the eyes are parallel. Paradoxic diplopia is demonstraed. (b); according to how the squint becomes manifest, coloured image moves towards the fixing light.

Normal correspondence in the anomalous correspondence cases. If anomalous correspondence was a congenital sensory anomaly as stated above and tf the patient had anomalous correspondence only, motor disturbance might occur since birth and although the deviation was corrected by operation, normal correspondence would not be restored. Most patients, however, may have both normal and anomalou scorrespondence at birth and therefore the squint may be intermittent as a rule. Moreover even if the squint became constant for some reason, normal correspondence should not fade.

In the anomalous correspondence cases, the patient who has an intermittent divergent squint possesses normal correspondence together and it is present normal and anomalous correspondence, whether the patient makes an effort to maintain binocular vision or abandon it. Normal correspondence was demonstrated not only in above cases, but also in 15 cases of constant exotropia and esotropia by the after-image test; it is shown that normal correspondence may be demonstrated in 70% of the total patients with anomalous correspondence before treatment.

The facts suggest that the vast majority of the anomalous correspondence cases possess normal correspondence together, which is difficult to be demonstrated before treatment, but easy after the eyes become parallel. Therefore restoration of normal correspondence by the treatment does not mean that retinal correspondence changes its type by an adaptation.

Summary

From the observations of 600 cases of concomitant squint anomalous correspondence could not be regarded as an adaptative phenomenon but as a congenital sensory anomaly.

1. A statistical analysis of a series of squints showed that in 239 cases with anomalous correspondence only 45 cases (20%) were found to be convergent and the other 194 cases (80%) to be divergent; moreover, intermittent squinting was found in 73% of the total cases of the divergent squint with anomalous correspondence, of which in 31% it was manifested with delayed onset after the 6th year.

2. There were fundamental differences between the normal and anomalous correspondence group. The striking features were the relationships between the type of correspondence and the intermittency, angle of the squint, and the cause of squint. In the vast majority of the cases with anomalous correspondence the angle of squint was found to be at 20 to 24 degrees and the cause of the squint was undetermined. There were also marked differences between two groups in the refractive curve. These findings suggest that the anomalous correspondence group is not derived from the normal correspondence group and that each group belongs to a different category in origin. In 15 cases of a divergent squint the hereditary tendency of anomalous correspondence was observed.

3. In 70% of the anomalous correspondence cases the presence of normal correspondence was demonstrated by the after-image test before treatment. Patients with anomalous correspondence have it with normal correspondence together in principle and therefore restoration of normal correspondence by treatment does not mean, that retinal correspnodence changes its type by an adaptation.

From the investigation of the visul function in the patients with anomalous correspondence it is condsidered that anomalous correspondence is the manifested result of congenital sensory anomaly, such as panoramic vision, that appears to be endowed

with conditions more dominant than the normal, and constitutes the cause of a squint.

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